

Gut inflammation can boost horizontal gene transfer between pathogenic and commensal *Enterobacteriaceae*

Bärbel Stecher^{a,b,1}, Rémy Denzler^a, Lisa Maier^a, Florian Bernet^a, Mandy J. Sanders^c, Derek J. Pickard^c, Manja Barthel^a, Astrid M. Westendorf^d, Karen A. Krogfelt^e, Alan W. Walker^c, Martin Ackermann^{f,g}, Ulrich Dobrindt^{h,i}, Nicholas R. Thomson^c, and Wolf-Dietrich Hardt^a

^aInstitute of Microbiology, ETH Zürich, 8093 Zürich, Switzerland; ^bMax-von-Pettenkofer Institute, Ludwig-Maximilians-Universität Munich, 80336 Munich, Germany; ^cWellcome Trust Sanger Institute, Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom; ^dInstitute of Medical Microbiology, University Hospital, University Duisburg-Essen, 45122 Essen, Germany; ^eDepartment of Surveillance and Research, Statens Serum Institut, 2300 S Copenhagen, Denmark; ^fDepartment of Environmental Sciences, ETH Zürich, 8092 Zürich, Switzerland; ^gDepartment of Environmental Microbiology, Eawag, 8600 Dübendorf, Switzerland; ^hInstitute for Molecular Infectious Biology, University of Würzburg, 97080 Würzburg, Germany; and ⁱInstitute for Hygiene, University of Münster, 48149 Münster, Germany

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The mammalian gut harbors a dense microbial community interacting in multiple ways, including horizontal gene transfer (HGT). Pangenome analyses established particularly high levels of genetic flux between Gram-negative *Enterobacteriaceae*. However, the mechanisms fostering intraenterobacterial HGT are incompletely understood. Using a mouse colitis model, we found that *Salmonella*-inflicted enteropathy elicits parallel blooms of the pathogen and of resident commensal *Escherichia coli*. These blooms boosted conjugative HGT of the colicin-plasmid p2 from *Salmonella enterica* serovar Typhimurium to *E. coli*. Transconjugation efficiencies of ~100% in vivo were attributable to high intrinsic p2-transfer rates. Plasmid-encoded fitness benefits contributed little. Under normal conditions, HGT was blocked by the commensal microbiota inhibiting contact-dependent conjugation between *Enterobacteriaceae*. Our data show that pathogen-driven inflammatory responses in the gut can generate transient enterobacterial blooms in which conjugative transfer occurs at unprecedented rates. These blooms may favor reassortment of plasmid-encoded genes between pathogens and commensals fostering the spread of fitness-, virulence-, and antibiotic-resistance determinants.

bacterial evolution | hospital-acquired infection | mucosal immune response | plasmid spread

The mammalian gut harbors a very dense microbial community, the “microbiota” ($>10^{12}$ bacteria/g), which has profound effects on the host’s nutrition, physiology, and immune system (1). In humans, the microbiota is generally composed of several hundred different bacterial phylotypes. Its composition differs between individuals forming a “collective microbiome” of more than 100,000 genes (2, 3). Much of the microbiota’s genome plasticity is thought to be attributable to horizontal gene transfer (HGT), the most effective mechanism of which is conjugation, the exchange of plasmids (4–6). Conjugational plasmid transfer is consistently fueling the emergence of hypervirulent or antibiotic-resistant pathogens (7, 8), as illustrated by *Escherichia coli* O104:H4, which has recently caused an outbreak in Germany (9). However, key questions about the maintenance and the transfer of conjugative plasmids in natural bacterial populations have remained unanswered. Here, we describe the discovery of a mechanism driving efficient conjugation between *Enterobacteriaceae* in the host’s intestine. *Enterobacteriaceae* include many pathogenic as well as commensal species (i.e., *Salmonella enterica* and *E. coli*). Plasmid profiling, *enterobacteriaceal* genome sequencing, and microbiome analyses identified a large and highly diverse plasmid-encoded accessory gene pool indicative of efficient conjugative HGT (10). However, in the normal gut, the *Enterobacteriaceae* are generally present in very low densities ($<<10^8$

cfu/g). These densities are way too low for efficient conjugative plasmid transfer, as this process hinges on direct physical contact between the donor and the acceptor bacterium (11).

In the mammalian intestine, the vast majority of intestinal bacteria consist of obligate anaerobic members of the Firmicutes and Bacteroidetes phyla. It is thought that these anaerobes keep the overall density of facultative anaerobic bacteria (i.e., *Enterobacteriaceae*) rather low ($<<10^8$ cfu/g), a condition termed colonization resistance (CR) (12–14). Thus, in complex bacterial ecosystems, low densities of donor and recipient bacteria may lower the frequency of direct bacterial encounters and thus decrease the chance of conjugation-mediated HGT. Several studies reported inefficient enterobacterial HGT in the normal mammalian gut (15–17), whereas others identified higher rates of HGT (18, 19). This suggested that particular conditions exist that might favor plasmid exchange between *Enterobacteriaceae*. The factors influencing the efficiency of conjugative HGT in this bacterial family are incompletely understood to date.

Inflammatory host responses triggered by the gut immune system (in inflammatory bowel disease patients) or by pathogens such as *Salmonella* spp. or pathogenic *E. coli* strains can suppress the anaerobic microbiota and boost enterobacterial colonization densities (20–24). Here, we show that disease-triggered enterobacterial blooms can fuel HGT between two prominent members of this family, *Salmonella enterica* serovar Typhimurium (*S. Tm*) and *E. coli*.

Results and Discussion

We have frequently observed parallel blooms ($\geq 10^8$ cfu/g each) of *S. Tm* strain SL1344 (*S. Tm*^{wt}) and commensal *E. coli* in our experiments using the streptomycin mouse model for *Salmonella* diarrhea. Fig. 1 shows the presence of commensal *E. coli* recorded during routine screening of *S. Tm*^{wt} infected mice by plating on selective agar (Fig. 1A) (21, 25, 26). In some cases, *E. coli* accounted for >80% of the total intestinal bacteria as

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¹To whom correspondence should be addressed. E-mail: stecher@mvp.uni-muenchen.de.

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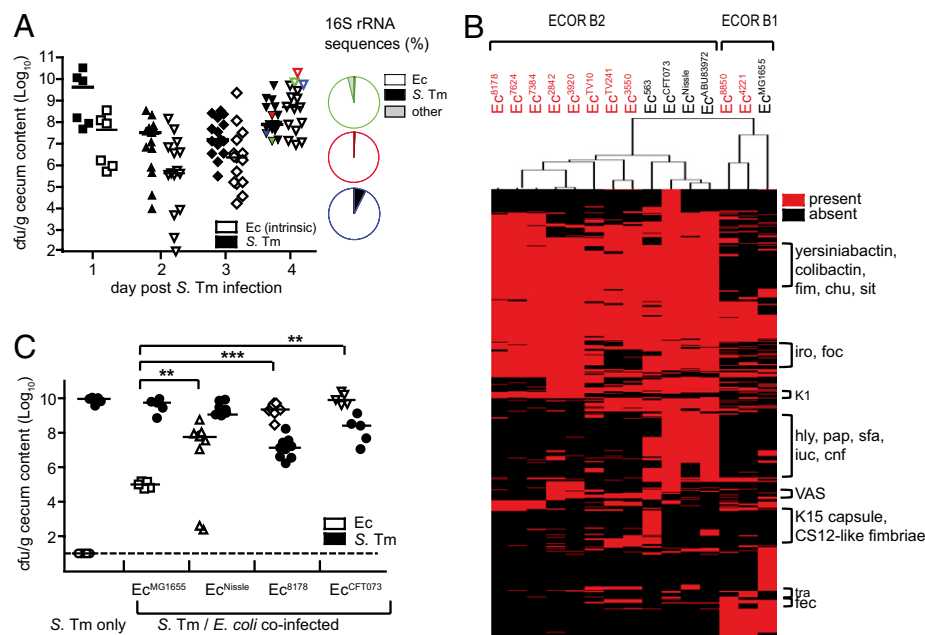


Fig. 1. Blooms of commensal *E. coli* in *S. Tm* infected mice. (A) Colonization levels of commensal *E. coli* in cecal contents of *S. Tm* infected mice. *E. coli* CFU were detected by routine screening on MacConkey without antibiotics by colony morphology and lactose-positive phenotype. Pie plots on the right show microbiota composition in three mice (marked in green, red, and blue). Here, total DNA was extracted, and bacterial 16S rRNA genes were PCR-amplified using universal bacterial primers, cloned, and sequenced (~100 sequences per animal). (B) DNA microarray comparing the virulence gene content of 10 *E. coli* isolates collected from *S. Tm* infected mice from 2002 to 2007 (indicated in red) and different pathogenic and nonpathogenic *E. coli* reference strains. Genes were grouped with the CLUSTER software based on the presence (red) or absence (black) of genes. Groups of genes belonging to distinct islands or phages are indicated. (C) Coinfection experiments of *S. Tm* and different *E. coli* strains. Streptomycin-treated, *E. coli*-free mice were coinfecting with 1:1 mixtures of wild type *S. Tm* and *E. coli* strains Ec^{MG1655}, or the ECOR B2 strains Ec^{Nissle}, Ec⁸¹⁷⁸ and Ec^{CFT073} (total of 5×10^7 ; 1:1; intragastrically). *E. coli* (white) and *S. Tm* (black) colonization density in the cecum at day 4 p.i. (Log₁₀ cfu/g). Bars show the median.

determined by 16S rRNA sequencing (Fig. 1A; red, green, and blue symbols at day 4 p.i.). Based on this result, we hypothesized that these high enterobacterial densities might favor HGT. Our aim was thus to investigate whether HGT had taken place between *Salmonella* and *E. coli* during these parallel blooms. We began our study by randomly isolating 10 “co-blooming” *E. coli* strains from *S. Tm* infected mice during the last 8 y in our laboratory (Table S1). Comparative genome analysis using an *E. coli* phylogenetic microarray (27) and multilocus sequence typing (MLST; Fig. 1B and Fig. S14) established that the *E. coli* isolates were genetically distinct and most isolates belonged to the phylogenetic group ECOR B2 (Table S1). We decided to focus more closely on one of the isolates and randomly choose the ECOR B2 strain Ec⁸¹⁷⁸ for further analysis. Ec⁸¹⁷⁸ was isolated from the animal depicted in further in Fig. 1A. *S. Tm* coinfection experiments in mice lacking intrinsic *E. coli* showed that Ec⁸¹⁷⁸ and two other ECOR B2 strains (Ec^{Nissle} and Ec^{CFT073}), but not Ec^{MG1655}, were as well adapted to growth in the inflamed gut (Fig. 1C and Fig. S1B). Interestingly, Ec⁸¹⁷⁸ also bloomed in mice suffering from T cell-induced gut inflammation, which had been demonstrated previously for *S. Tm* (Fig. S2; ref. 21). Identifying the genetic traits underlying adaptation to growth in the inflamed gut will be an interesting topic for future research.

To uncover possible events of HGT, we generated a shotgun genome sequence of Ec⁸¹⁷⁸. Analysis of the contigs showed that Ec⁸¹⁷⁸ harbored about 1 Mbp extra DNA compared with Ec^{MG1655} and that the closest sequenced relative was the uropathogenic strain Ec^{CFT073} (28). However, the strain Ec⁸¹⁷⁸ was negative for all tested typical virulence factors of pathogenic *E. coli* (Table S2). Curiously, Ec⁸¹⁷⁸ harbored an 86-kb plasmid >99% identical to plasmid 2 (termed p2) from *S. Tm* strain SL1344 (<http://www.sanger.ac.uk/resources/downloads/bacteria/salmonella.html>), which is routinely used for infection studies by

us and by others (Fig. 2A). P2 shows homology to R-64 and Collb-P9-type conjugative plasmids of the IncI1 incompatibility group, a family of plasmids also encoding multiple antibiotic resistances (29–31) (Fig. 2B). However, p2 from *S. Tm*^{wt} was by far the closest relative of the plasmid found in Ec⁸¹⁷⁸. The only difference between the two plasmids was an inversion in the shufflon region encoding the variable pilus tip antigen (30) (Fig. 2A and Fig. S3). Shufflon rearrangements are frequently observed and are thought to adapt sex pilus binding specificity to the new recipient strain (32). We were concerned that modification of the shufflon region in Ec⁸¹⁷⁸ p2 may have disrupted the pilus tip antigen essential for conjugative HGT. However, control experiments verified that the plasmid had retained its ability to conjugate into various *E. coli* and *S. enterica* strains, albeit at various efficiencies (Table S3).

These observations suggested that p2 was acquired by conjugative HGT during the infection experiment. In line with this hypothesis, *S. Tm*^{wt} p2 carries a complete set of genes required for conjugative transfer. Furthermore, plasmids of similar size and structure, as revealed by gel electrophoresis, PCR and Southern blot, were detected in 4 of the 10 *E. coli* isolates from *E. coli*-blooms in *S. Tm*^{wt}-infected mice (Fig. 2C and Fig. S4).

It should be mentioned that the C57BL/6 mouse colony used for *Salmonella* infection experiments is maintained under strict barrier conditions, monitored regularly for the absence of pathogens, and kept separate from the facility where the infection experiments are performed, making previous contact of Ec⁸¹⁷⁸ to pathogens as *S. Tm*^{wt} highly unlikely. This strongly suggested that p2 was indeed only acquired during the course of our infection experiments, i.e., by HGT from *S. Tm*. The fact that 4 of 10 independent *E. coli* isolates harbored this plasmid (Fig. 2C) provided a first hint that conjugative HGT might occur at

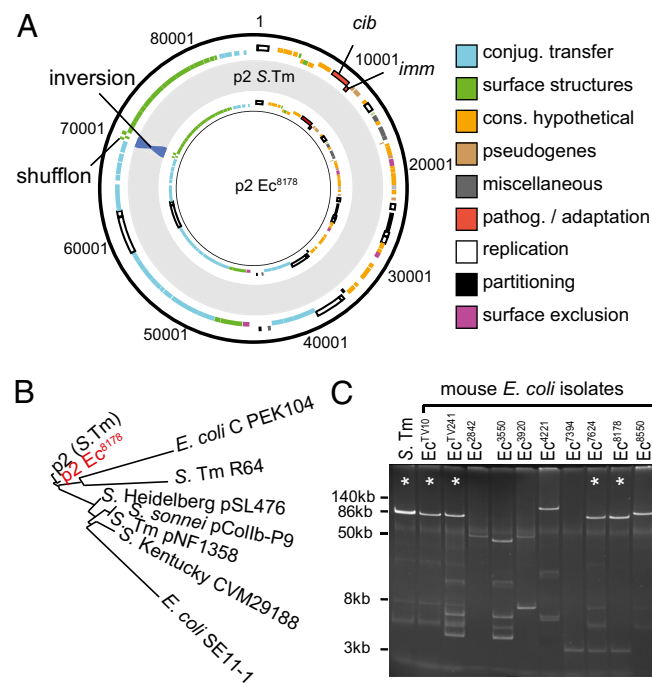


Fig. 2. Highly similar conjugative plasmid is present in *S. Tm* and commensal *E. coli*. (A) *S. Tm* and *Ec*⁸¹⁷⁸ share a nearly identical conjugative plasmid. Sequence comparison of p2 *S. Tm* (outer circle) and p2 *Ec*⁸¹⁷⁸ (inner circle); gene functions are color coded. (B) Phylogram showing relation of p2 to other R-64 and Colb-P9-type conjugative plasmids (*E. coli* PEK104, *S. Tm* R64-1, *S. Tm* R64-2, *S. Heidelberg* pSL476, *Shigella sonnei* pColb-P9, *S. Tm* pNF1358, *S. Kentucky* CVM29188, *E. coli* SE11-1). The phylogram was generated using PHYLIB and the Clustal W multiple sequence alignment algorithm; branch length represents the relative nucleotide differences of each plasmid compared with the reference sequence p2. (C) p2 is detected by gel electrophoresis in 4 of 10 *E. coli* isolates collected from *S. Tm*-infected mice (from Fig. 1B).

extremely high rates if donor (*S. Tm*) and recipient (commensal *E. coli*) bloom in parallel.

The emergence of transconjugants might be affected by two different phenomena: plasmid-encoded fitness-factors favoring transconjugant growth and by the efficiency of the conjugation process itself. We have engineered appropriate plasmid mutants and bacterial strains to investigate the importance of either mechanism.

First, we analyzed the contribution of plasmid-encoded fitness factors. P2 contains a locus for colicin Ib production (*cib*) and immunity (*imm*) (Fig. 2A). Colicins are toxic proteins produced by and toxic for bacteria of the *Enterobacteriaceae* family (33, 34). Colicin-producing bacteria protect themselves from colicin-dependent killing by the expression of a cognate immunity protein. In the case of colicin Ib (Cib), the cognate immunity protein inserts into the inner bacterial membrane thus blocking Cib-mediated pore formation and killing (33). Thus, p2 might enhance bacterial fitness by killing of competing *Enterobacteriaceae* lacking the plasmid (e.g., *Ec*^{Nissle}). Indeed, we found evidence for this hypothesis in vitro and in vivo. *S. Tm*^{wt} produced colicin Ib in vitro. Colicin Ib of *S. Tm*^{wt} killed susceptible *E. coli* strains such as *Ec*^{MG1655} and *Ec*^{Nissle} (Table S4). Killing was ablated if *S. Tm* harbored a *cib imm* deletion on p2 (*S. Tm* p2^{Δcib}).

Similarly, we analyzed colicin Ib and Imm function in vivo. To this end, we chose *Ec*^{Nissle} because it is sensitive to colicin Ib and naturally resistant to HGT (35). Mouse coinfection experiments comparing *Ec*^{Nissle} vs. *S. Tm* growth in *S. Tm*^{wt} or *S. Tm*^{wt} p2^{Δcib} infected mice demonstrated that colicin Ib production conferred a small, but significant competitive advantage in the gut ($P < 0.05$; Fig. 3A). In conclusion, colicin Ib production (*cib*) represents

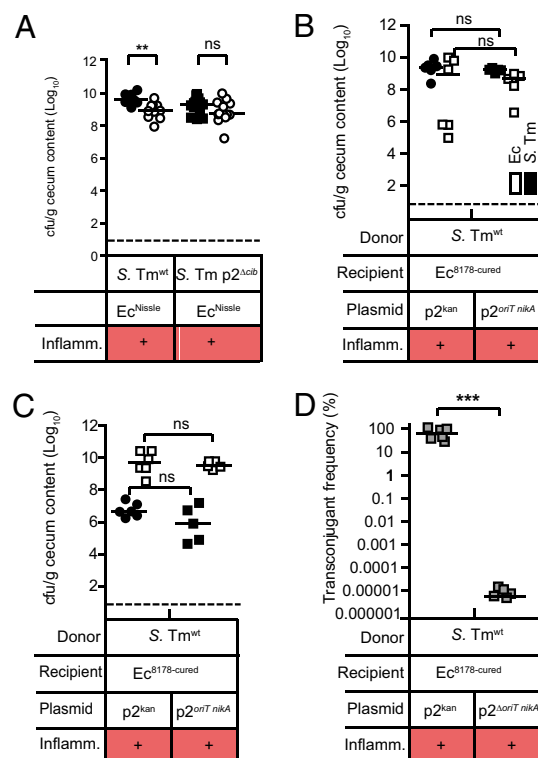


Fig. 3. Conjugative *Salmonella* plasmid can confer a fitness benefit and is efficiently transferred to commensal *E. coli* in vivo. (A) Colicin Ib production leads to superior growth of *S. Tm* in competition against *Ec*^{Nissle} in the gut. Streptomycin-treated mice were coinfecting with 1:1 mixtures (total of 5×10^7 ; 1:1; intragastrically) of *Ec*^{Nissle} and *S. Tm*^{wt} or *S. Tm*^{wt} p2^{Δcib}, respectively. Transfer of p2 into *Ec*^{Nissle} could not be detected, which is in line with the established resistance against HGT of this strain. B, C. P2 is transferred to *Ec*⁸¹⁷⁸-cured by conjugation with high efficiency. Streptomycin-treated mice were coinfecting with 1:1 mixtures (total of 5×10^7 ; 1:1; intragastrically) of *Ec*⁸¹⁷⁸-cured and *S. Tm*^{wt} carrying p2^{kan} or p2^{ΔoriT nika}, respectively. Densities of the *S. Tm* donor (black) and *E. coli* recipient strains (white) were determined in the feces at day 1 p.i. (B) and in the cecal contents at day 4 p.i. (C), and frequency of kanamycin-resistant *E. coli* transconjugants in cecal contents was determined by selective plating at day 4 p.i. (D). *E. coli* (white) and *Salmonella* (black) colonization densities (Log₁₀ cfu/gram) were determined in the feces at day 1 p.i. (B) or cecal content at day 4 p.i. (A and C). Bars show the median.

a fitness factor that may enhance the apparent transconjugant frequencies observed in the *Salmonella*-infected mouse gut.

Next, we analyzed the in vivo conjugation efficiency of the *S. Tm* p2. To measure the conjugation efficiency in the absence of *cib*-mediated fitness effects, we generated a p2-cured variant of *Ec*⁸¹⁷⁸ as a recipient (*Ec*⁸¹⁷⁸-cured, Fig. S4 and Table S2). Interestingly, this strain is insensitive to colicin Ib (Table S4). We speculate that this is due to spontaneous second site mutations in the chromosome, i.e., in the genes for colicin Ia binding and internalization: *cirA*, *tonB*, and *exbCD* (33). However, the precise location of this putative mutation could not be identified by comparison of the respective genes from resistant and sensitive strains so far. Anyhow, *Ec*⁸¹⁷⁸-cured is a highly efficient recipient for conjugative transfer of a derivative of p2 (p2^{kan}) genetically tagged with an antibiotic resistance marker (Table S3). The marker affected neither Cib production nor immunity of the donor strain.

To measure conjugative HGT in vivo, we coinfecting streptomycin-treated mice with *Ec*⁸¹⁷⁸-cured (recipient) and *S. Tm* p2^{kan} (donor). Both strains bloomed to high density in the infected murine intestine (Fig. 3B and C). Strikingly, the transconjugant frequency was extremely high, yielding almost 100% of

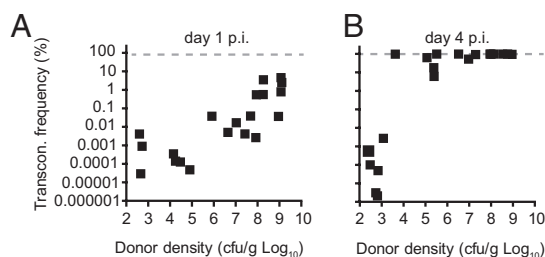


Fig. 5. Intestinal density of *S. Tm* plasmid donors correlates with *E. coli* transconjugant frequency. Streptomycin-treated conventional mice ($n = 20$) were coinoculated with mixtures of *Ec*^{8178, cured amp} (recipient; amp^R), *S. Tm*^{tet} p2^{Δcib} (donor; tet^R, plasmid p2: kan^R) and increasing amounts of *S. Tm*^{wt} p2^{ΔoriTnikAacib} (competitor; cm^R) at no ($n = 4$), 10-fold ($n = 3$), 100-fold ($n = 3$), 1,000-fold ($n = 3$), 10,000-fold ($n = 3$), and 100,000-fold ($n = 4$) excess over the donor strain (total of 5×10^7 ; intragastrically). The density of the *S. Tm* donor (x axis) was plotted against *E. coli* transconjugant frequencies (fraction of amp^R kan^R *E. coli* of total amp^R *E. coli*; y axis) as determined in the feces at day 1 p.i. (A) and cecal contents at day 4 p.i. (B). A linear relationship between cfu and transconjugant frequency could be predicted by linear regression analysis ($P < 0.05$).

those typically encountered in the normal mammalian intestine (14, 38). This fuels the reassortment of genetic material between different *Enterobacteriaceae* and suggests that infected patients might enhance the spread of plasmid-encoded fitness-, virulence- and antibiotic resistance-determinants (e.g., extended-spectrum β -lactamases; ref. 39). Our findings shift the current paradigm of the “separate” evolution of pathogens (i.e., *Salmonella*) and commensals (i.e., *E. coli*): Both are interconnected: Episodes of pathogen-inflicted disease boost not only the evolution of pathogens, but also that of the commensals. Our findings might be of central relevance for explaining the notorious emergence of new pathogenic and commensal strains with altered phenotypes.

Finally, our data may add to the question of how plasmids are maintained in bacterial populations over evolutionary time (“plasmid persistence puzzle”; see *SI Text* for detailed discussion) (40). Based on theoretical considerations, carriage of beneficial genes is insufficient to account for plasmid maintenance. Due to fitness costs, plasmids should be lost and beneficial genes should move to the chromosome. To explain the paradox of plasmid existence, several mechanisms had been proposed: high-rate conjugative HGT (“infectious transmission”), plasmid shuttling between bacterial strains, ecotypes or species (40) and the existence of “amplifier strains,” exhibiting extraordinarily high transconjugation efficiencies (41). However, so far, the evidence supporting these hypotheses was mostly limited to theoretical models and in vitro data. Our analysis of p2 transfer in the infected gut provides evidence that “infectious transmission” as well as plasmid shuttling between bacterial strains contribute to plasmid maintenance in nature. This must occur at very high frequencies, as 4 of 10 independent strains analyzed in our initial experiments had acquired p2 (Fig. S4). Thus, plasmid transfer in enterobacterial blooms comprising different bacterial species is most likely a frequent event. Pathogenesis-inflicted blooms may be an important feature of enterobacterial plasmid biology.

Materials and Methods

Bacteria and Plasmids. Bacterial strains and plasmids used are listed in Table S2. For infections, *E. coli* and *S. Tm* strains were grown as described (42). Generation of bacterial mutants and plasmids, *E. coli* phylogenetic microarray, genome sequencing, sequence comparisons and alignments, broad-range bacterial 16S rRNA gene sequence analysis and plasmid isolation procedures are described in *SI Materials and Methods*.

Isolation and Characterization of *E. coli* Strains From Frozen Tissue. Sections of cecum tissues embedded in O.C.T. (Sakura) were prepared on a previously

sterilized cryotome. Sections were suspended in PBS and live bacteria recovered by plating on MacConkey agar plates. Lactose-positive colonies were further characterized using the Enterotube system (Becton Dickinson). ECOR typing was performed using a well established triplex-PCR (43). MLST analysis was done as described by sequencing of the *adh*, *fumC*, *gyrB*, *ica*, *mdh*, *purA*, *recA*, and *ST* gene fragments and corresponding allocation to sequence types (STs).

Animal Experiments. All mice used in the study were on C57BL/6 background and bred at the Rodent Center at the ETH Zürich under SPF conditions in individually ventilated cages. LCM mice colonized with the Altered Schaedler flora were described (26). Conventional, *E. coli*-free mice (CON) were initially obtained from Janvier, bred at the RCHCI and routinely tested for the absence of *E. coli* in the feces. For *S. Tm* infections, mice were treated with streptomycin (20 mg per animal 24 h before *Salmonella* infection) and infected by gavage with 5×10^7 cfu *S. Tm* or mixtures of *S. Tm* and *E. coli* as described. Live bacterial loads in the cecal content were determined by plating on MacConkey-agar (Oxoid) with respective antibiotics (streptomycin 100 μ g/mL; kanamycin 30 μ g/mL and chloramphenicol 30 μ g/mL; ampicillin 100 μ g/mL; tetracycline 12 μ g/mL). Histology was done at necropsy. Briefly, tissues were embedded in O.C.T. (Sakura) and flash frozen. H&E-stained cecum cryosections were scored as described: Evaluating submucosal edema, PMN infiltration, goblet cells, and epithelial damage yielded a total score of 0–13 points.

For induction of acute colitis, CD8⁺ T cells from CL4-TCR transgenic mice that express an α / β T cell receptor recognizing an epitope of the HA protein presented by MHC class I (the H-2Kd:HA512-520 complex) were adoptively transferred into VILLIN-HA mice that express the A/PR8/34 HA epitope from influenza virus A under control of the enterocyte-specific villin promoter (44). Single-cell suspensions were prepared from the spleen of CL4-TCR transgenic mice. Cell suspensions were depleted of CD4⁺, CD11b⁺, CD45R⁺, DX5⁺, and Ter-119⁺ cells by using the MACS CD8 T cell isolation kit (Miltenyi Biotec). CL4-TCR T cells were purified by negative selection according to the manufacturer's instructions. Isolated CD8⁺ T cells were washed once in PBS and resuspended (4×10^7 cells per mL of PBS). Then, 4×10^6 purified CL4-TCR transgenic T cells were injected i.v. into VILLIN-HA transgenic mice. Disease symptoms (weight loss and diarrhea) were observed 4–5 d after adoptive transfer. Animal experiments were approved (licenses 201/2004 and 201/2007 Kantonales Veterinäramt Zürich) and performed according to local guidelines (TschV, Zurich) and the Swiss animal protection law (TschG).

Immunofluorescence. For detecting *S. Tm* and *Ec*⁸¹⁷⁸ in the gut lumen in situ, cecal tissues were recovered and treated as described (45). Briefly, the tissue was fixed in paraformaldehyde (4% in PBS, pH 7.4 overnight, 4 °C), washed with PBS, equilibrated in PBS (20% sucrose, 0.1% Na₂S₂O₄ overnight, 4 °C), embedded in O.C.T. (Sakura), snap-frozen in liquid nitrogen and stored at –80 °C. Cryosections (7 μ m) were air-dried for 2 h at room temperature, fixed in 4% paraformaldehyde (5 min), washed, and blocked in 10% (wt/vol) normal goat serum in PBS for 1 h. *S. Tm* and *Ec*⁸¹⁷⁸ were detected by staining for 1 h with a polyclonal rabbit α -*Salmonella*-O-antigen group B serum (factors 4, 5; Difco; 1:300) or with a polyclonal rabbit α -*E. coli*-O7 serum (Statens Serum Institut; 1:100) and DyLight-549-conjugated secondary goat- α -rabbit antibody (Jackson). DNA was stained with Sytox-green (1:10,000; Invitrogen). Sections were mounted with Vectashield hard set (Vector Laboratories) and analyzed using a Leica SP5 confocal laser scanning microscope (Leica Microsystems).

Statistical Analysis. Statistical analysis was performed using the exact Mann-Whitney *U* test (Graphpad Prism Version 5.01). *P* values less than 0.05 (two-tailed) were considered statistically significant.

Colicin Assay. For measuring production of colicin and colicin sensitivity, the colicin-producing strain was grown overnight as small spot (6 mm) on LB agar containing 2 μ g/mL Mitomycin C (Sigma). The plate was overlaid with the tester strain in Top-agar (0.75% agarose). Growth of the tester strain was analyzed after 24 h. Halo formation around the producer indicated sensitivity of the tester strain.

Mating Experiments. An overnight culture of donor and recipient strains was grown in LB media with appropriate antibiotics (37 °C, 180 rpm). The recipient strain was marked with antibiotic resistance by either carrying pVK530 (46) (Amp) or pACYC184 (New England Biolabs; Cm). A total of 1 mL of each overnight culture was washed three times in PBS and concentrated (300 μ L PBS final). Donor and recipient strains were mixed at a ratio of 1:1. Eighty microliters of the mixture was spread on LB Agar plate (surface mating), and matings were incubated for 4 h at 37 °C. Bacteria were recovered and plated onto respective selective agar to quantify transconjugant frequencies (cfu of plasmid-positive/total recipient cfu).

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