Effect of *Caenorhabditis elegans* age and genotype on horizontal gene transfer in intestinal bacteria

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ABSTRACT **Horizontal gene transfer (HGT) between bacteria occurs in the intestinal tract of their animal hosts and facilitates both virulence and antibiotic resistance. A model in which both the pathogen and the host are genetically tractable facilitates developing insight into mechanistic processes enabling or restricting the transfer of antibiotic resistance genes. Here we develop an *in vivo* experimental system to study HGT in bacteria using *Caenorhabditis elegans* as a model host. Using a thermosensitive conjugative system, we provide evidence that conjugation between two *Escherichia coli* strains can take place in the intestinal lumen of N2 wild-type worms at a rate of 10^{-3} and 10^{-2} per donor. We also show that *C. elegans* age and genotype are important determinants of the frequency of conjugation. Whereas ~1 transconjugant for every 100 donor cells could be recovered from the intestine of N2 *C. elegans*, for the *age-1* and *tol-1* mutants, the detected rate of transconjugation (10^{-3} and 10^{-4} per donor cell, respectively) was significantly lower. This work demonstrates that increased recombination among lumenal microbial populations is a phenotype associated with host aging, and the model provides a framework to study the dynamics of bacterial horizontal gene transfer within the intestinal environment.—Portal-Celhay, C., Nehrke, K., Blaser, M. J. Effect of *Caenorhabditis elegans* age and genotype on horizontal gene transfer in intestinal bacteria. *FASEB J.* 27, 760–768 (2013). www.fasebj.org**

Key Words: conjugation · mating · in vivo

Horizontal gene transfer (HGT) is an ancient phenomenon that affects the entire biosphere (1). Of particular importance is microbial HGT, which has a continuing strong influence on evolution (2–4) and is a model to understand evolution of more complex organisms. For microbes that interact with host species, HGT plays important roles shaping these interactions (5–8). However, although pioneering work began in the 1960s (9–11), the effect of animal host environments on HGT between bacteria has been little-studied over the years (12). Gene exchange that may be limited under *in vitro* conditions may readily occur *in vivo* in environments to which the microbes have evolved, and in response to host signals. The gut in different animal species, including humans, may be an important milieu for gene transfer, since large and diverse microbial communities reside there (13, 14).

Residential bacteria not only exchange antibiotic-resistance genes among themselves (15), but also interact with transient bacteria, which leads to the bidirectional transfer of resistance genes (16). Bacteria colonizing the intestine of laboratory animals have been observed to transfer antibiotic resistance genes under particular circumstances (17–20). Antibiotic resistance genes can be disseminated among bacterial populations by transduction, transformation, or by conjugation. A model in which both the pathogen and the host are genetically tractable facilitates developing insight into mechanistic processes enabling or restricting the transfer of antibiotic resistance genes. As such, we developed an *in vivo* experimental system to study horizontal gene transfer in bacteria, using the nematode *Caenorhabditis elegans* as a model host.

When *C. elegans* ingest bacteria as their food source, a proportion remains viable and can colonize and proliferate in its intestinal tract (21, 22). Specific *C. elegans* innate immune pathways regulate intestinal bacterial proliferation, via expression of antimicrobial peptides (23, 24), and differences in pH (25) as well as differing concentrations of antimicrobial peptides in the intestinal milieu (26) also could affect conjugation rates. In this study, we assess the frequency of conjugation of *Escherichia coli* in the intestinal lumen of *C. elegans* wild-type and mutant worms. Since both *E. coli* and *C. elegans* are genetically defined and tractable, this approach permits identifying factors that regulate HGT between bacteria *in vivo*.

Abbreviations: HGT, horizontal gene transfer; LB, Luria-Bertani; mNGM, modified nematode growth medium; RAPD, random amplified polymorphic DNA

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MATERIALS AND METHODS

C. elegans strains and growth conditions

All strains were provided by the *Caenorhabditis* Genetic Center (University of Minnesota, Minneapolis, MN, USA)and maintained on modified (0.35% peptone) nematode growth mediuma (mNGM), using standard procedures (27). *E. coli* DY330 [Tet^R (efflux pump Tn10), Str^S], which contains a derepressed form of plasmid R27 (the prototypical IncHI1 conjugative plasmid), and *E. coli* OP50 (Str^R chromosomal marker, Tet^S), were kindly provided by Diane E. Taylor (University of Alberta, Edmonton, AB, Canada; ref. 28) and Fred M. Ausubel (Harvard Medical School, Boston, MA, USA), respectively. Bacterial cultures were grown in Luria-Bertani (LB) broth at 37°C, supplemented with either tetracycline (20 μg/ml) or streptomycin (60 μg/ml). **Table 1** indicates the *C. elegans* and bacterial strains used in this study.

In vitro conjugation

Bacterial conjugation was achieved *in vitro* by mixing *E. coli* strains DY330/(donor)/(OP50)/(recipient). After incubation at different temperatures, the bacterial mixture was plated onto antibiotic-containing selective medium [LB agar supplemented with either streptomycin (60 μg/ml), tetracycline (20 μg/ml), or both] that allowed identification of donor, recipient, and potential transconjugant strains.

In vivo conjugation assay

To examine *in vivo* bacterial conjugation, *C. elegans* embryos were grown on mNGM agar plates seeded with *E. coli* DY330 (donor strain; see **Fig. 1*A***). Based on our preliminary studies (29, 30), at d 4 of adulthood, worms were washed to remove surface bacteria and transferred to mNGM agar plates seeded with *E. coli* OP50 (recipient strain). After 24, 48, or 72 h, 10 worms were washed, homogenized by grinding, and plated on selective agars, as above, to quantify the three distinct bacterial populations. Conjugation frequency was estimated by dividing the number of transconjugants per worm by the number of donor bacteria per worm. All mating experiments were conducted at 25°C.

Confirmation of *in vitro* conjugation by random amplified polymorphic DNA (RAPD)-PCR

To determine whether conjugation actually occurred, and to rule out spontaneous mutations conferring Str^R in the donor strain, transconjugants were characterized by RAPD-PCR fingerprinting (31). Colonies were isolated from agar plates selective for the resistance phenotype (Tet^R Str^R) of putative transconjugants, and genomic DNA was extracted and purified, followed by PCRs with RAPD primers 1254 (5′-CCGCAGCCAA-3′) or 1290 (5′-GCGGAAATAG-3′), using conditions as described previously (32). Template DNA from the donor and recipient strains was included in the PCR as positive controls to indicate strain origin of the putative transconjugants.

Measurement of *C. elegans* intestinal pH

The vital dye Oregon Green 488 Dextran (1 μM) was fed to N2 and mutant *C. elegans* in S medium supplemented with *E. coli* OP50 for 1 h at room temperature. The worms then were pipetted onto an NGM-agarose plate for imaging. Image acquisition, analysis, and calibration were performed as described previously (33), with dual excitation wavelengths of 490 and 440 nm.

Statistical analysis

Transconjugant populations and conjugation frequencies in wild-type *C. elegans* and mutants were compared using 2-sample *t* tests assuming equal variances; values of $P < 0.05$ were considered significantly different from control.

RESULTS

Temperature-sensitive transfer of H incompatibility group plasmid *in vitro*

The wild-type *C. elegans* N2 strain could be cultivated at temperatures between 15 and 25°C (34). To determine whether *C. elegans* can be used as a model host to study HGT, a conjugation system that can transfer DNA at these temperatures was required. The ability to transfer

TABLE 1. C. elegans *and* E. coli *strains used in this study*

Strain	Relevant phenotype	Source and references
C. elegans		
N2	Wild type, reference *C. elegans* strain	*Caenorhabditis* Genetic Center (University of Minnesota, Minneapolis, MN, USA)
daf-16 (mu86)I	Decreased lifespan; decreased resistance to heat, oxidative stress, and pathogens	40
age-1 (hx546)II	Extended lifespan; increased resistance to heat, oxidative stress, and pathogens	40
tol-1 (nr2033)I	Unable to avoid pathogenic bacteria; susceptible to killing by gram-negative bacteria	41,42
phm-2 (ad597)I	Defective terminal bulb; allows greater numbers of intact bacteria to enter the intestinal tract	43
E. coli		
OP50mgh	Uracil auxotroph, Str^R	Fred M. Ausubel (Harvard Medical School, Boston, MA, USA)
DY330 with plasmid drR27	Rif^R, Tet^R, Km^R	Diane E. Taylor (University of Alberta, Edmonton, AB, Canada)

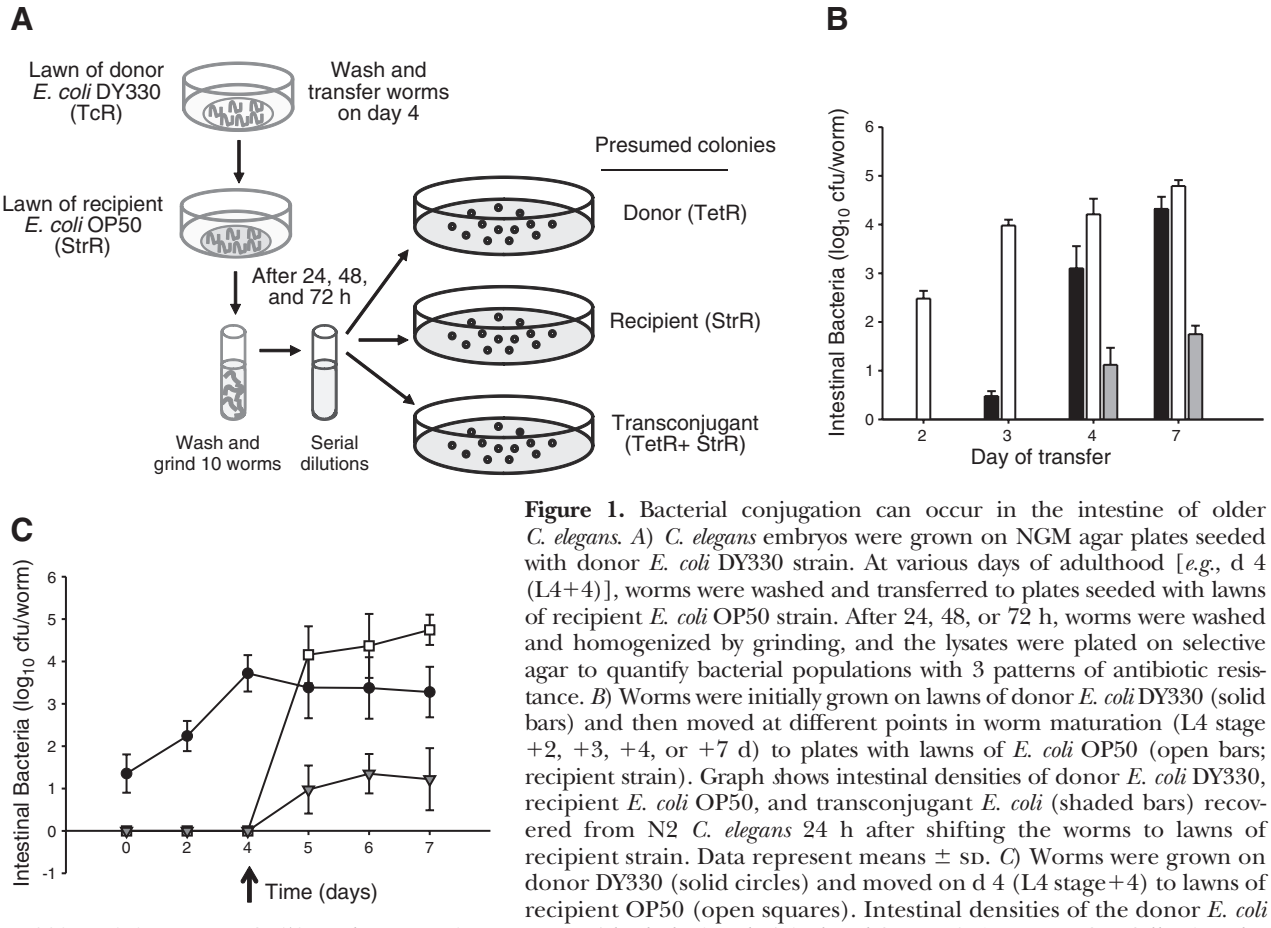

Figure 1. Bacterial conjugation can occur in the intestine of older *C. elegans*. *A*) *C. elegans* embryos were grown on NGM agar plates seeded with donor *E. coli* DY330 strain. At various days of adulthood [*e.g.*, d 4 (L4+4)], worms were washed and transferred to plates seeded with lawns of recipient *E. coli* OP50 strain. After 24, 48, or 72 h, worms were washed and homogenized by grinding, and the lysates were plated on selective agar to quantify bacterial populations with 3 patterns of antibiotic resistance. *B*) Worms were initially grown on lawns of donor *E. coli* DY330 (solid bars) and then moved at different points in worm maturation (L4 stage +2, +3, +4, or +7 d) to plates with lawns of *E. coli* OP50 (open bars; recipient strain). Graph shows intestinal densities of donor *E. coli* DY330, recipient *E. coli* OP50, and transconjugant *E. coli* (shaded bars) recovered from N2 *C. elegans* 24 h after shifting the worms to lawns of recipient strain. Data represent means ± SD. *C*) Worms were grown on donor DY330 (solid circles) and moved on d 4 (L4 stage+4) to lawns of recipient OP50 (open squares). Intestinal densities of the donor *E. coli* DY330, recipient *E. coli* OP50, and transconjugant *E. coli* (shaded triangles) isolated from N2 *C. elegans* after following the same worm population over the 3 d following transfer are shown. Data represent means ± SD.

DNA between 15 and 25°C was first studied *in vitro* to provide an indication of the likelihood of genetic transfer occurring within worms raised at that temperature. We tested a self-transmissible resistance plasmid (drR27) belonging to the IncHI1 plasmid group (35), which has temperature-sensitive conjugative transfer. These plasmids have predominantly been identified in *Enterobacteriaceae* (36) and play a central role in the emergence and reemergence of bacterial pathogens, since they encode resistance to multiple antibiotics (37, 38).

After 24 h of mating at 20°C and 25°C, donor *E. coli* strain DY330, which carries pdrR27, showed rates of transfer of 10^{-3} and 10^{-2}, respectively, to recipient *E. coli* strain OP50 (data not shown). In contrast, transfer at 37°C was negligible, confirming previous observations that showed that IncHI1 plasmids transfer optimally between 22 and 30°C (39). From these results, we conclude that pdrR27 transfer can be examined *in vivo* in *C. elegans*, since its occurrence would be predicted to be a relatively frequent event at temperatures at which *C. elegans* can be cultivated.

Bacterial gene transfer in the intestine of *C. elegans*

Next, we asked whether exchange of genetic material among *E. coli* strains could occur within the *C. elegans* intestinal tract. The *C. elegans* wild-type strain N2 was maintained and propagated on donor strain *E. coli* DY330 using standard techniques. Then worms were transferred to lawns composed of recipient strain *E. coli* OP50 and then sampled for transconjugants on several days after transfer to determine which day of worm life was optimal to perform the conjugation assay (Fig. 1*A*). As increasingly older worms were exposed to the introduced recipient *E. coli* strain (OP50), progressively greater intestinal persistence of the donor *E. coli* strain DY330 was found. When worms were transferred on d 2 of adulthood, only recipient *E. coli* OP50 could be isolated from the intestine. However, when transfer was done later, bacterial densities of persisting donor strains within the *C. elegans* intestine increased significantly ($P<0.001$), with smaller concomitant increases in the introduced recipient strain (Fig. 1*B*). These results are consistent with previous observations, indicating that worm age determines the persistence of founder bacteria (29). Under the conditions tested, putative bacterial conjugation was detected and increased significantly as the worms aged. As a control, we harvested and plated all bacteria growing on the NGM plates. Finding only cells of the recipient strain (*E. coli* OP50) ruled out the possibility of gene transfer outside the worms and of worms feeding on transconjugants.

Based on the results from these experiments, we selected transfer at d 4 as a condition for further study.

To further test the ability of conjugation to proceed *in vivo*, we next investigated the persistence of donor, recipient, and putative transconjugant cells in the *C. elegans* intestine. In these experiments, *C. elegans* were grown on lawns of *E. coli* donor DY330 and transferred to the recipient *E. coli* strain OP50 on adult d 4 (Fig. 1*C*, arrow), and then groups of worms were sampled 1 to 3 d after the transfer. As shown in Fig. 1*C*, the recipient strain introduced on d 4 rapidly colonized the intestine with 10^4 to 10^5 cfu/worm, as expected (29). Although no further introduction of the donor strain was starting on d 4, it also persisted in the intestine of the worms, at stable levels of $\sim 10^3$ cfu/worm. Strains with both antibiotic resistances could be detected in the intestinal contents 24 h after the recipient strain was introduced, at stable concentrations of $\sim 10^1$ cfu/worm.

Confirmation of *in vivo* conjugation by RAPD-PCR

To confirm that conjugation had occurred and to rule out spontaneous mutations conferring streptomycin resistance in the donor strain, putative transconjugants isolated from the plates containing both of the antibiotics (Tet and Str) on which selection was based were characterized by RAPD-PCR fingerprinting. DNA from the donor and recipient strains was included in the PCR as positive controls to indicate strain origin of the putative transconjugants. RAPD-PCR assays revealed band profiles that clearly distinguished between the donor and recipient strains (**Fig. 2**); 2–3 major polymorphic bands were amplified with each of the primers. In the experiment shown, four putative transconjugant isolates with the expected phenotype were examined, and the results show that all had RAPD-PCR profiles identical to those of the recipient strain, as expected, since spontaneous mutation conferring resistance to streptomycin are rare. Based on these findings, we conclude that conjugation had been achieved and that the double-resistant phenotype of transconjugants results from DNA transfer from the expected donor to recipient strain.

Role of the host intestinal milieu in bacterial gene transfer

We next examined the role of variation within the intestinal milieu within *C. elegans* on the rate of bacterial gene transfer. We studied *age-1* and *daf-16* worms that have essentially opposite phenotypes, restricting and enhancing bacterial colonization in the intestinal lumen, respectively (30, 40). We also examined mutants in *tol-1,* the sole Toll-like receptor (TLR) in *C. elegans*, which are required for worms to avoid pathogenic bacteria (41) and for the expression of ABF-2, which is a defensin-like molecule expressed in the pharynx of the worms, and of HSP-16, which is involved in stress resistance (42). Finally, we examined *phm-2* mutants, which have an abnormal pharyngeal grinder (43), and therefore increased delivery of living bacterial cells to the intestine. In all four *C. elegans* mutants, sufficient members of both donor and recipient *E. coli* cells could persist in the intestine after the introduction

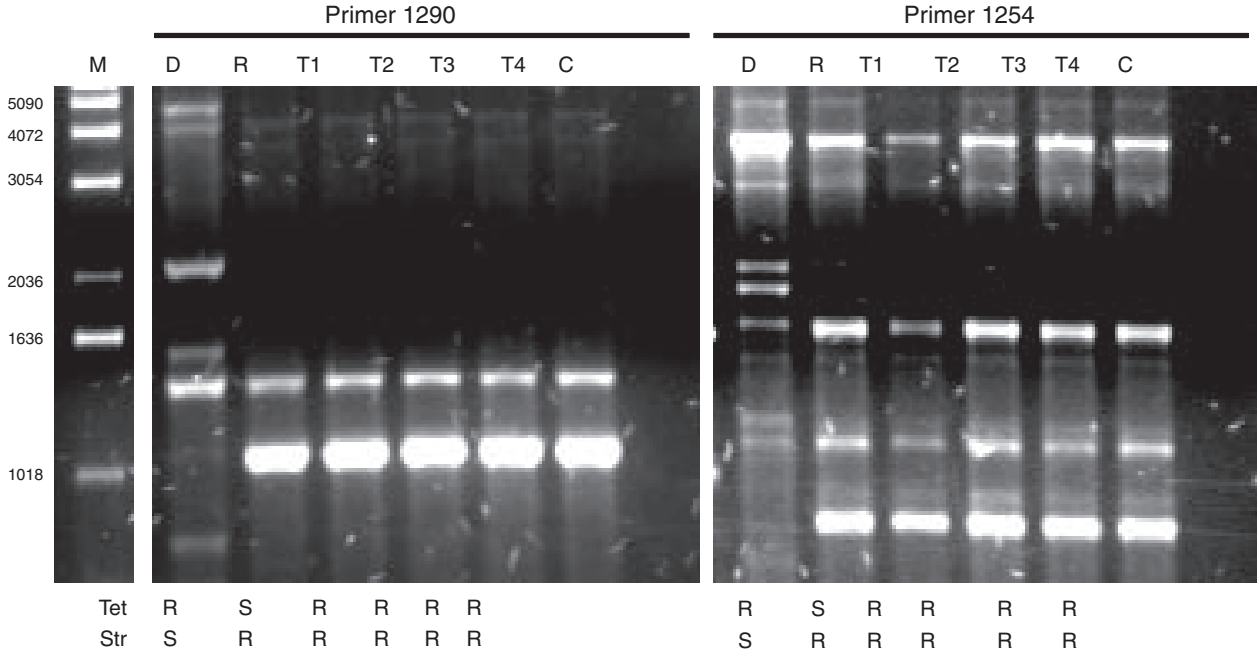

Figure 2. RAPD profiles of donor, recipient, and putative transconjugant strains. The donor *E. coli* DY330 (letter D) recipient *E. coli* OP50 (R) and 4 putative transconjugants (T1–T4) were studied by RAPD. Amplifications were performed using primer 1290 (left panel) or primer 1254 (right panel). Lane at left contains a 1-kb molecular size marker (M); lanes C represent the negative controls. Labels at bottom indicate the antibiotic-resistance phenotype of the strains (S, susceptible; R, resistant).

of the recipient strain on d 4 to allow transfer (**Fig. 3**); however, each worm genotype showed different bacterial densities and kinetics of colonization (Fig. 3). In the *age-1* mutants, donor *E. coli* DY330 intestinal counts were low (~10 cfu/worm) at d 4, but after the introduction of the recipient strain increased to concentrations of 10^3 on each subsequent day (Fig. 3*A*). This increase represents intraintestinal proliferation, since the worms no longer had DY330 cells in their external environment. In contrast, *phm-2* mutants had ~10^5 viable donor *E. coli* DY330 cells/worm on d 4, but concentrations decreased ~1 log after transfer to lawns with recipient strain *E. coli* OP50 (Fig. 3*D*); this decrease represents the effects of competition between the founder (donor) and the introduced (recipient) bacteria. Intestinal concentrations of the donor strain in the *daf-16* and *tol-1* mutants were maintained similar to N2 wild-type worms, despite no further introduction (Fig. 3*B, C*). The introduced recipient strain colonized the intestines of all four mutants to similar extents as with N2 (10^4 to 10^5 cfu/worm; Fig. 3). Thus, in both the wild-type and mutant worms, cocolonization of the intestinal lumen by both donor and recipient strains was achieved. In each worm background, transconjugation had occurred (Fig. 3).

Role of host genotype on conjugation frequencies

Although transconjugants were detected in all four mutants by the day after the introduction of the recipient strain and colonized the intestine throughout the experiment, patterns differed by host genotype (**Fig. 4*A***, top panel). The trends became most apparent by d 6. A progressive increase was found in the number of transconjugants until d 6 in the N2, *phm-2*, and *daf-16* mutants. However, the number of intestinal transconjugants in the *age-1* and *tol-1* mutants did not rise and remained significantly ($P<0.005$) lower than in the N2 worms (Fig. 4*A*, bottom panel).

As expected, each worm genotype showed different donor bacterial densities at the day of transfer (d 4); however, after the introduction of the recipient strain, the donor concentrations (on d 6) were nearly the same in all five worm genotypes (Fig. 4*B*). We next examined the frequency of conjugation in relation to the number of donor cells. At 24 h after transfer (d 5), the calculated conjugation rates of the bacteria within the intestine of N2 and mutants were very similar (Fig. 4*C*, top panel); however, by 48 h (d 6) after the introduction of the recipient strain, significant differences in frequencies were observed (Fig. 4*C*,

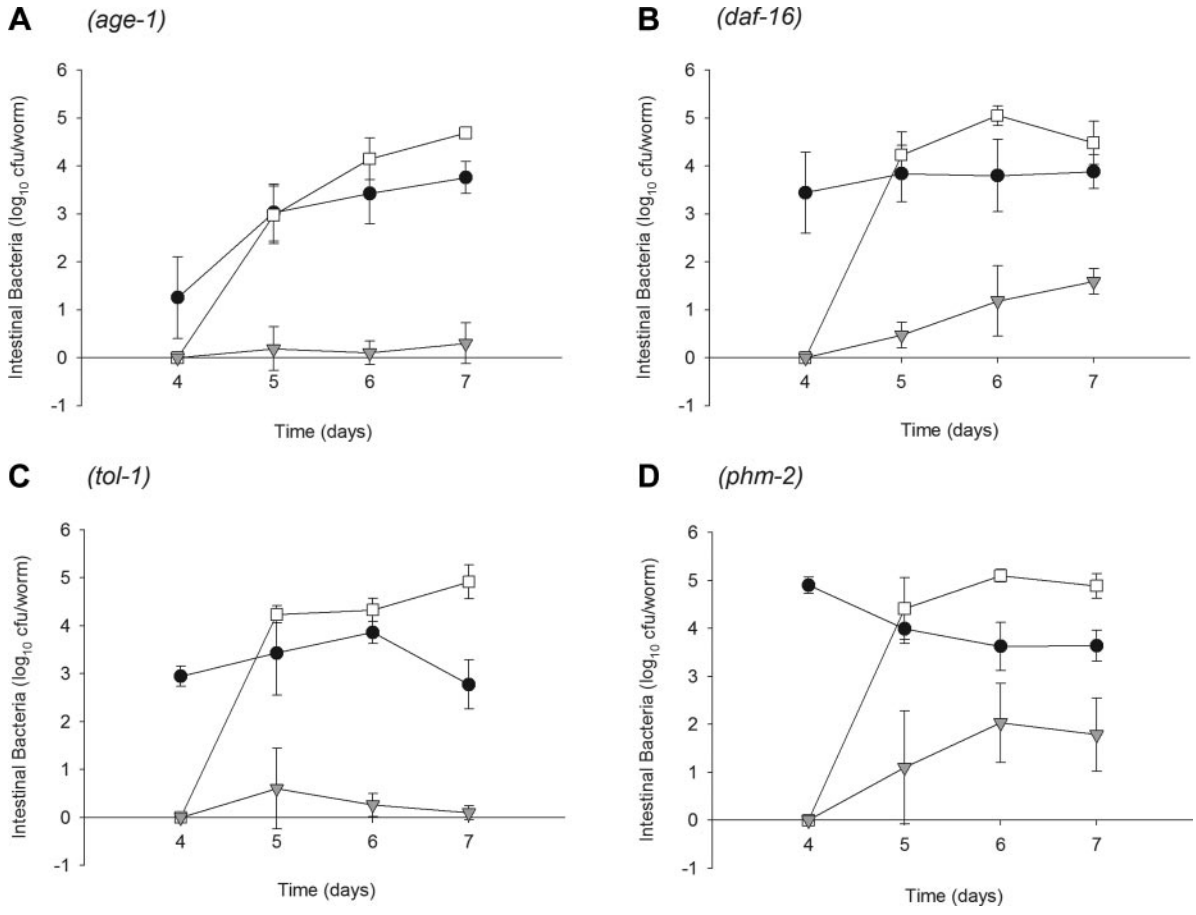

Figure 3. Bacterial conjugation in the intestinal lumen of *C. elegans* mutants. Intestinal densities of donor *E. coli* DY330 (solid circles), recipient-*E. coli* OP50 (open circles), and transconjugant *E. coli* (shaded triangles) isolated from: *age-1* (*A*); *daf-16* (*B*); *tol-1* (*C*); or *phm-2* (*D*) mutants. Worms were grown on *E. coli* DY330, and then on d 4 shifted to lawns of *E. coli* OP50.

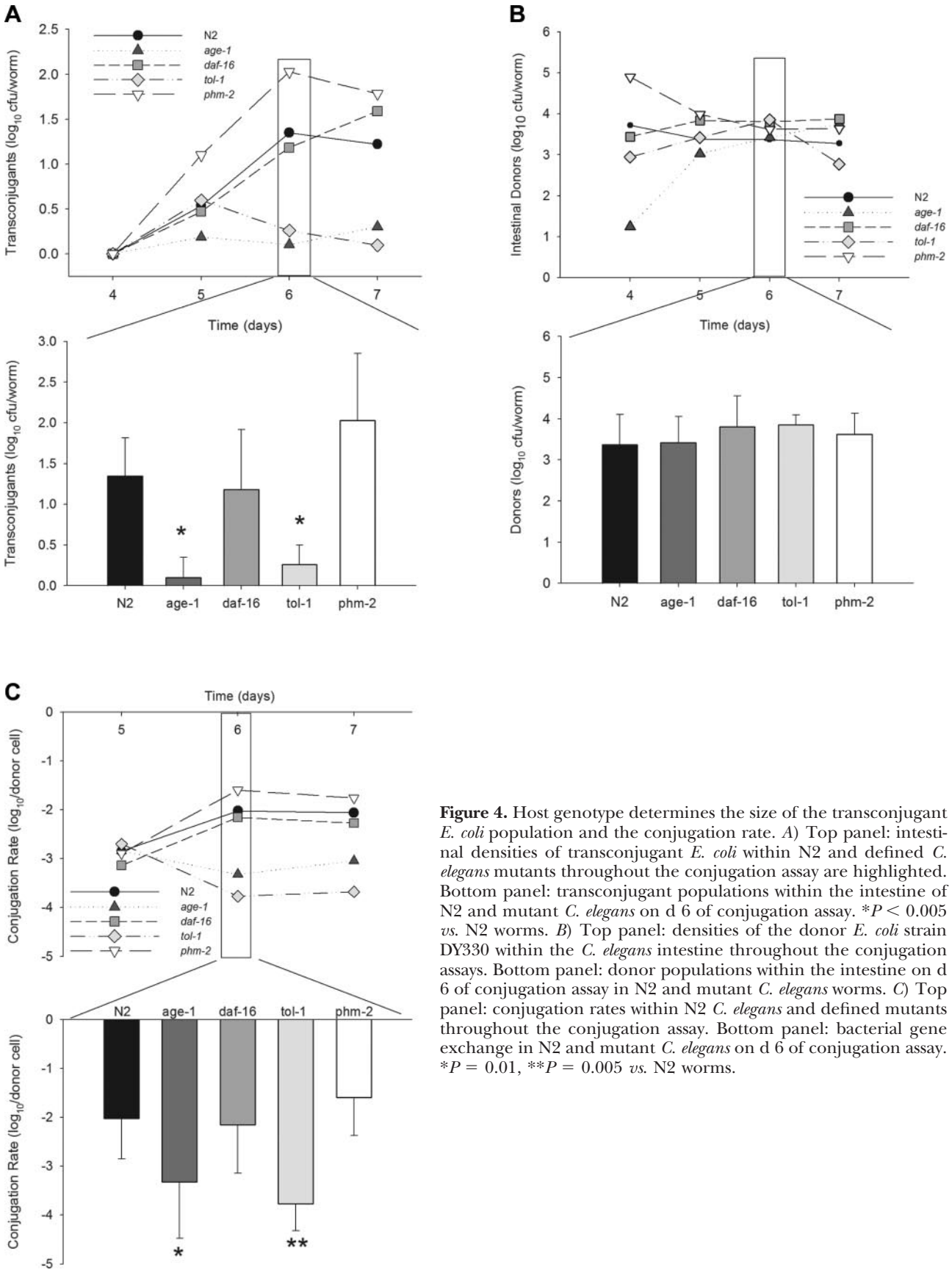

Figure 4. Host genotype determines the size of the transconjugant *E. coli* population and the conjugation rate. *A*) Top panel: intestinal densities of transconjugant *E. coli* within N2 and defined *C. elegans* mutants throughout the conjugation assay are highlighted. Bottom panel: transconjugant populations within the intestine of N2 and mutant *C. elegans* on d 6 of conjugation assay. $*P < 0.005$ *vs.* N2 worms. *B*) Top panel: densities of the donor *E. coli* strain DY330 within the *C. elegans* intestine throughout the conjugation assays. Bottom panel: donor populations within the intestine on d 6 of conjugation assay in N2 and mutant *C. elegans* worms. *C*) Top panel: conjugation rates within N2 *C. elegans* and defined mutants throughout the conjugation assay. Bottom panel: bacterial gene exchange in N2 and mutant *C. elegans* on d 6 of conjugation assay. $*P = 0.01$, $**P = 0.005$ *vs.* N2 worms.

bottom panel). Whereas ~1 transconjugant for every 100 donor cells could be recovered from the intestine of N2 (wild-type) *C. elegans*, and from the *phm-2* and *daf-16* mutants, for the *age-1* and *tol-1* mutants, the detected rate of transconjugation (10^{-3} and 10^{-4} per donor cell), respectively, was significantly lower than for the N2 wild-type worms (Fig. 4*C*, bottom panel). Thus, *C. elegans* genotype clearly affects genetic exchange in the intestinal lumen, with lowest rates in *age-1* and *tol-1* worms.

Effect of intestinal pH on conjugation rates

Among factors known to affect the rate of plasmid transfer in bacterial cells are pH (44–46), temperature (39, 44, 47), nutrients (48, 49), and bacterial density (50, 51). The intestinal pH of *C. elegans* might vary according to genetic background and, consequently, could affect conjugation rates in the lumen. To test this hypothesis, we measured the intestinal pH of the mutants by feeding them dextran conjugated to the pH-sensitive vital dye Oregon Green-488 in S-basal medium for 1 h, followed by live fluorescent imaging (25, 33). Although the pH in the intestinal lumen of *C. elegans* oscillates during defecation (25, 52), each of the tested mutants had similar luminal pH levels (Supplemental Fig. S1), which were consistent with the intestinal pH of wild-type worms described previously (25). Thus, intestinal pH is well-conserved in these mutants, and therefore, luminal pH variation cannot explain the different transconjugation frequencies observed.

DISCUSSION

HGT has had an enormous effect on bacterial biodiversity and evolution (53). HGT in the intestinal environment of animals is of particular interest, due to its contribution to the stability of the microbiota, emergence of new pathogens and to antimicrobial-resistant strains (54–56). Using a temperature-sensitive conjugative plasmid (39), we now show that *C. elegans* can be used as a model system to study HGT *in vivo* in the intestinal tract, and that host genotype affects the phenomenon.

The high numbers of both donor and recipient bacteria cocolonizing the intestine of 4-d-old adult *C. elegans* allowed mating to occur at detectable levels. Higher ratios of transconjugants per donor *in vivo* compared to *in vitro* assays has been reported (48, 57), suggesting that *in vitro* systems underestimate the transfer potential; however, the opposite relationship also has been observed (58). In our study, conjugation frequencies obtained in the N2 worms ranged between 10^{-3} and 10^{-2} per donor, similar or higher than the *in vitro* transfer rates of 1×10^{-3} transconjugants/donor. The ability of the donor strain to persist in the *C. elegans* intestine is necessary for conjugation to occur; high donor density increases encounters of donors and recipients, increasing mating events (59). In previous studies, we found a strong correlation between bacterial counts and life span (30), and we created a model system to distinguish between continuing accumulation *vs.* bacterial proliferation (29). We found that the host's age, as well as bacterial strain, determines the nature of bacterial persistence in the *C. elegans* intestine. Using microscopy and strains labeled with fluorescence, we confirmed dual colonization. Our work also provided evidence for active competition *in vivo* for colonization sites, as well as evidence for *in vivo* bacterial adaptation (29). Thus, aging in *C. elegans*, which permits intestinal persistence of founding colonizing strains (Fig. 3 and ref. 29), facilitates intraluminal genetic transfer. This is a new phenotype associated with aging in *C. elegans*.

However, transfer frequency events do not solely depend on bacterial density. The nematode intestine is dynamic, and bacterial mating *in vivo* may be more complex than mating in *vitro*. Since environmental factors have the potential to affect the frequency and outcome of conjugative gene transfer, by studying *C. elegans* mutants, we could evaluate roles of particular host genotypes in gene transfer. We found no significant differences in conjugation rates between the *phm-2* mutants and the *daf-16* mutants compared to wild-type (Fig. 4*B*) despite differences in numbers of bacteria delivered to the intestine (*phm-2*) and in their ability to colonize the intestine (*daf-16*) (30). The significantly lower rates of conjugation in *age-1* and *tol-1* mutants also do not reflect intestinal levels of bacterial colonization (Fig. 4*B*) but must reflect variation in the host intestinal milieu. It is possible that increased levels of antimicrobial peptides and antioxidant enzymes are responsible of the decreased HGT observed in *age-1* mutants. However, lower conjugation rate phenotype was surprising for *tol-1* mutants, which have decreased expression of *abf-2* (42), an insect like-defensin with proven antimicrobial properties, and of *hsp-16.41*, which is highly expressed in the worms pharynx in response to stress and belongs to a family of heat-shock proteins recently found to be required for *C. elegans* immunity (60). Since higher levels of HGT had been expected, other compensating defense molecules may have been up-regulated.

There is no single optimum pH for conjugation. However, conjugal transfer is affected by pH. In general, extremes of pH reduce the rate of transfer; this inhibition is more marked under acidic conditions than under alkaline conditions and is more significant as temperature decreases (44). Although differences in intestinal luminal pH do not appear to play a role in this phenomenon (Supplemental Fig. S1), other luminal factors, such as the content of reactive oxygen species (ROS), antimicrobial peptides, or intestinal physical defenses, such as peristaltic movements, may be significant. In the future, studies using animals deficient in antioxidant enzymes such as *ctl-2* and *sod-3*, antimicrobial peptides such as lysozymes and caenopores (61), or defecation mutants (62) should be done.

Increases in the numbers of transconjugants, such as

observed between d 4 and 6 in the N2, *phm-2*, and *daf-16* worms (Fig. 4*A*), may represent either ongoing conjugation due to the continuing contact of mating strains or could represent proliferation of transconjugants in the intestinal lumen. While the studies we performed were not designed to differentiate between these two possibilities, the altered kinetics in the *age-1* and *tol-1* worms argues against the latter hypothesis. Similarly, although it is possible that the transconjugants are more fit in the intestinal milieu than their parents, this is unlikely since the introduced marker was not selected for *in vivo*. That the density of the transconjugants reached a relative plateau is consistent with that notion. However, the clear evidence of proliferation of the donor strain inside the *C. elegans* lumen may serve as a model for the dynamics (proliferation) of the transconjugants. The increase in the level of transconjugants over time observed in the *daf-16* mutants could result from the lack of antioxidant enzymes and/or antimicrobial peptides in the intestinal milieu (59), factors that might inhibit the process of transfer. Experiments designed to confirm this hypothesis, such as testing a *daf-16;age-1* double mutant and transformation rescue should be performed, as well as the development of a conditional GFP reporter to indicate *in vivo* mating.

Overall, our results indicate that we can reproducibly achieve bacterial conjugation in the intestinal lumen of a living organism (*C. elegans*) that is both genetically defined and experimentally tractable. We also provide evidence that both the age of the host and its genotype play roles in the *in vivo* regulation of bacterial gene transfer. The *C. elegans* intestinal lumen model system of microbial colonization, competition, and persistence provides a powerful tool that should lead to a better understanding of both host and microbial factors that affect horizontal gene transfer *in vivo*. FJ

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