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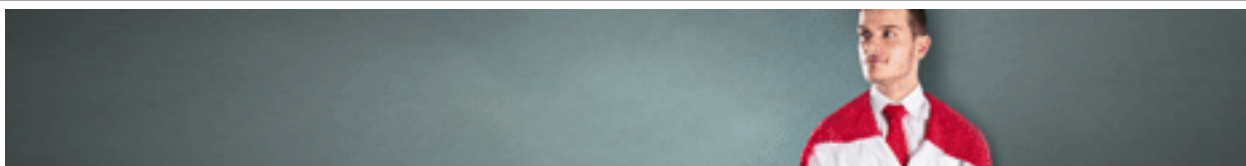
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# Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways

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We have determined the complete genome sequences of a host-promiscuous *Salmonella enterica* serovar Enteritidis PT4 isolate P125109 and a chicken-restricted *Salmonella enterica* serovar Gallinarum isolate 287/91. Genome comparisons between these and other *Salmonella* isolates indicate that *S. Gallinarum* 287/91 is a recently evolved descendent of *S. Enteritidis*. Significantly, the genome of *S. Gallinarum* has undergone extensive degradation through deletion and pseudogene formation. Comparison of the pseudogenes in *S. Gallinarum* with those identified previously in other host-adapted bacteria reveals the loss of many common functional traits and provides insights into possible mechanisms of host and tissue adaptation. We propose that experimental analysis in chickens and mice of *S. Enteritidis*-harboring mutations in functional homologs of the pseudogenes present in *S. Gallinarum* could provide an experimentally tractable route toward unraveling the genetic basis of host adaptation in *S. enterica*.

[Supplemental material is available online at [www.genome.org](http://www.genome.org). The genome sequence data from this study have been submitted to EMBL under accession nos. AM933172 and AM933173.]

Zoonotic pathogens, particularly those associated with veterinary animals in the human food chain, are some of the most important causes of infectious diseases in humans. Pathogens associated with zoonotic infections exhibit a promiscuous phenotype in that they maintain the ability to colonize and potentially cause infections in more than one host species. In contrast, some pathogenic agents are significantly host restricted, or adapted, and are normally only able to cause disease in one host. *Salmonella enterica* is a single bacterial species that includes examples of both promiscuous and host-adapted pathotypes. Iso-

lates from serovars such as *S. enterica* serovar Typhimurium and *S. Enteritidis* predominantly retain the ability to infect more than one mammalian host, including humans, whereas serovars such as *S. enterica* serovars Typhi and *S. Gallinarum* are restricted to humans and chickens, respectively. The ability to transmit between and within particular host populations is centrally important in dictating the epidemiology of infections and the emergence of new diseases.

Before the mid-1980s, *S. Enteritidis* was regarded as an *S. enterica* serovar of minor public health significance, but subsequently this serovar became dominant in terms of human food poisoning in many parts of the world (Rodrigue et al. 1990). National and international legislation regarding the reporting of disease incidence, improved hygiene and biosecurity (Barrow

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2000), and vaccination have contributed to controlling *S. Enteritidis* levels in poultry and consequently in man in Europe, but levels of infection remain significant. Most recent isolates of *S. Enteritidis* are regarded as promiscuous in the sense that they can cause infections in mice, retain the ability to colonize the tissues of chickens, and cause gastroenteritis in man.

*S. Gallinarum*, the causative agent of fowl typhoid, is a predominantly avian-restricted serovar (Shivaprasad 2000). Interestingly, in common with the human-restricted serovar *S. Typhi*, the chicken-adapted *S. Gallinarum* causes an invasive typhoid-like disease. Thus, here host adaptation appears to have co-evolved with loss of the intestinal lifestyle and the acquisition of the ability to cause systemic infection. *S. Gallinarum* still causes a disease of worldwide economic significance, and although it has been largely controlled in countries with strong health control policies, largely through serology-based test and slaughter schemes, it remains a problem elsewhere. Multi locus enzyme electrophoresis analyses of isolates of *S. Enteritidis* and *S. Gallinarum* indicate that, together with isolates of *S. Dublin* and *S. Pullorum*, they form a related strain cluster that share the same lipopolysaccharide-based O structure (O-1, 9, 12 characteristic of serogroup D). The nonmotile *S. Gallinarum* and *S. Pullorum* were previously suggested to have split independently from a motile ancestor related to *S. Enteritidis* (Li et al. 1993; McMeechan et al. 2005). Nonmotility in *S. Gallinarum* has been partially attributed to mutations in the flagellin subunit gene *fliC* gene (Kilger and Grimont 1993), which would normally express the phase 1 g, m antigens characteristic of *S. Enteritidis*. Nonmotility may enhance the ability to invade systemically from the gut by avoiding the TLR-5-induced pro-inflammatory responses of the host (Kaiser et al. 2000; Iqbal et al. 2005).

Here we report the full genome sequences of representative isolates of *S. Enteritidis* and *S. Gallinarum* and provide a detailed comparative genomic analysis of the two serovars. These data have been used to provide insight into the biology, mechanisms of host/tissue adaptation, and evolutionary relationships of these important pathogens.

## Results and Discussion

### General features of the *S. Enteritidis* PT4 strain P125109 and *S. Gallinarum* strain 287/91 genomes

The complete genome sequences of the promiscuous *S. Enteritidis* PT4 strain P125109 (hereafter *S. Enteritidis* PT4; EMBL accession no. AM933172) and the highly host-adapted chicken patho-

gen *S. Gallinarum* strain 287/91 (hereafter *S. Gallinarum* 287/91; EMBL accession no. AM933173) were determined and annotated. The main features are summarized in Table 1 and Figure 1, where they are compared with *S. Typhimurium* strain LT2 (hereafter *S. Typhimurium* LT2) (McClelland et al. 2001). The most striking feature of the analysis is the predominant similarity and synteny of core regions of the genomes, including many of the *Salmonella* pathogenicity islands (SPI). Indeed, this comparative analysis highlights an extremely close relationship between the genomes of *S. Enteritidis* and *S. Gallinarum*, suggesting the latter is a direct evolutionary descendent of the former. However, in comparison to *S. Enteritidis* PT4, *S. Gallinarum* 287/91 harbors a significantly higher number of predicted pseudogenes. Although the number of pseudogenes in *S. Enteritidis* PT4 is slightly higher than reported for *S. Typhimurium* LT2, it is in line with levels described in other broad host range enteric pathogens such as *Yersinia enterocolitica* (Thomson et al. 2006). In contrast, the number of pseudogenes in *S. Gallinarum* 287/91 is closer to that of the human-restricted *S. Typhi* CT18 (204 pseudogenes) (Parkhill et al. 2001) and *S. enterica* serovar Paratyphi A (173 pseudogenes) (McClelland et al. 2004).

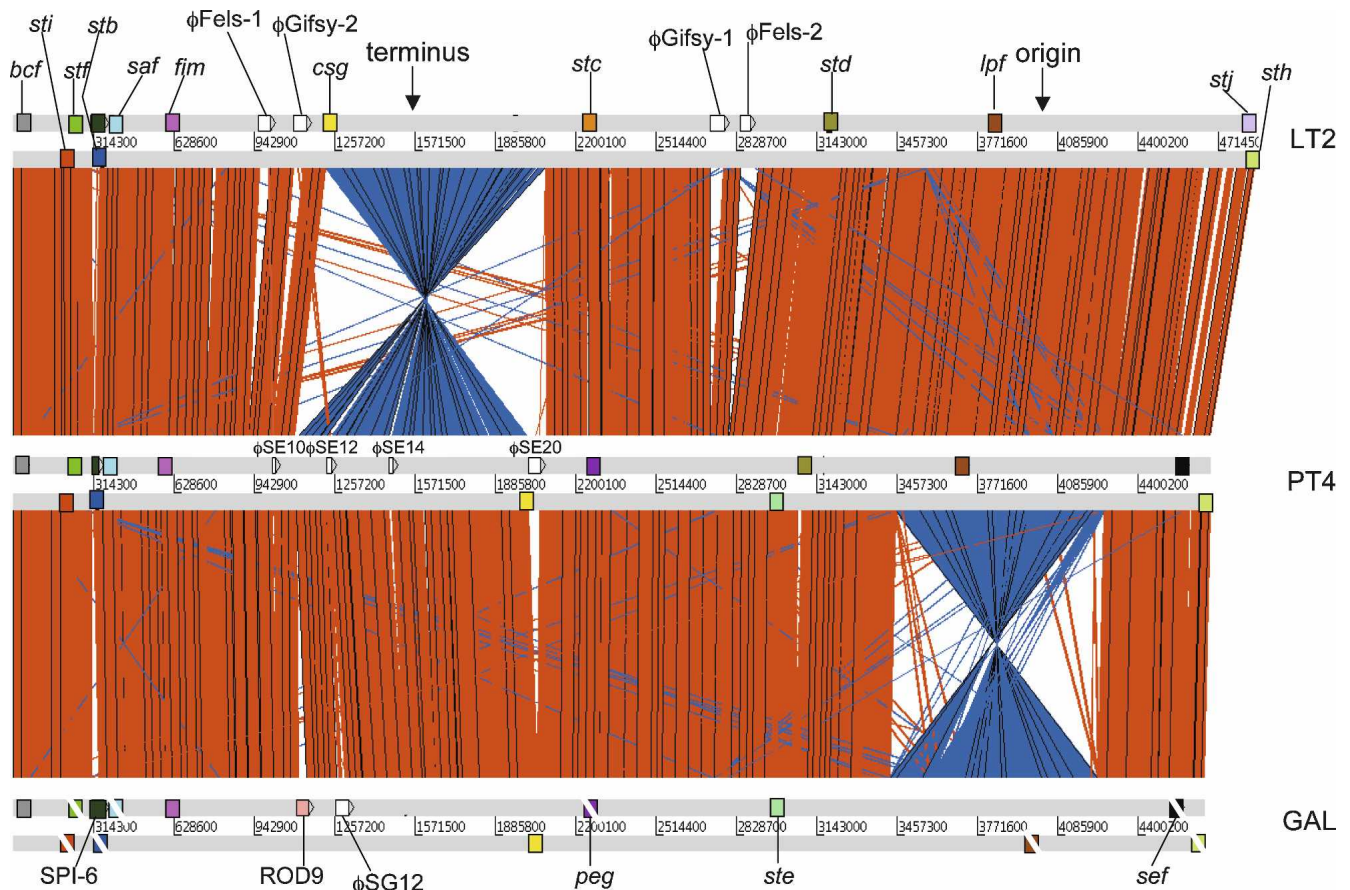
### Whole-genome comparisons of *S. Enteritidis* PT4 and *S. Typhimurium* LT2

Initially, the genome of *S. Enteritidis* PT4 was compared with that of *S. Typhimurium* LT2, a well-characterized and fully sequenced *S. enterica* isolate. *S. Enteritidis* PT4 and *S. Typhimurium* LT2 are both representatives of serovars able to cause enteritis in a broad range of hosts and produce murine typhoid, but they also show significant phenotypic differences, including serovar type. An alignment of the genome of *S. Enteritidis* PT4 with that of *S. Typhimurium* LT2 revealed colinearity except for an inversion about the terminus in *S. Typhimurium* LT2 (Fig. 1) (McClelland et al. 2001), with >90% of coding sequences (CDS) forming an extensive core gene-set (Figs. 2, 3). The average nucleotide identity between the shared orthologs is 98.98% compared with 99.7% between those of LT2 and a second fully sequenced *S. Typhimurium* strain SL1344 (data not shown). The genes that are only present either in *S. Enteritidis* PT4 or *S. Typhimurium* LT2 form 6.4% and 9.6% of their respective genomes (Fig. 3). The majority of *S. Enteritidis* PT4 unique CDS are in clusters from >3 kb up to >40 kb, but there are very few indels of <3 kb (Fig. 2; Table 2). We refer to these nonshared gene clusters as regions of difference (ROD). CDS present in *S. Enteritidis* PT4 but absent from *S. Typhimurium* LT2 are dominated by prophage-related functions, although other functional classes are represented (Fig. 3).

**Table 1.** General properties of *S. enterica* serovar genomes

Serovar	<i>S. enterica</i> serovars			
	Enteritidis	Typhimurium	Gallinarum	Typhi
Strain	P125109 (PT4)	LT2	287/91	CT18
Size	4,685,848	4,857,432	4,658,697	4,809,037
Percent G + C content (%)	52.17	52.22	52.20	52.09
No. of CDS	4318	4451	4274	4599
Coding density	85.5%	86.8%	79.9%	87.6%
Average gene size	953	947	939	958
rRNA operons	7	7	7	7
tRNA	84	85	75	78
Pseudogenes <sup>a</sup>	113	25	309	204

<sup>a</sup>Taken from original publications (see text).



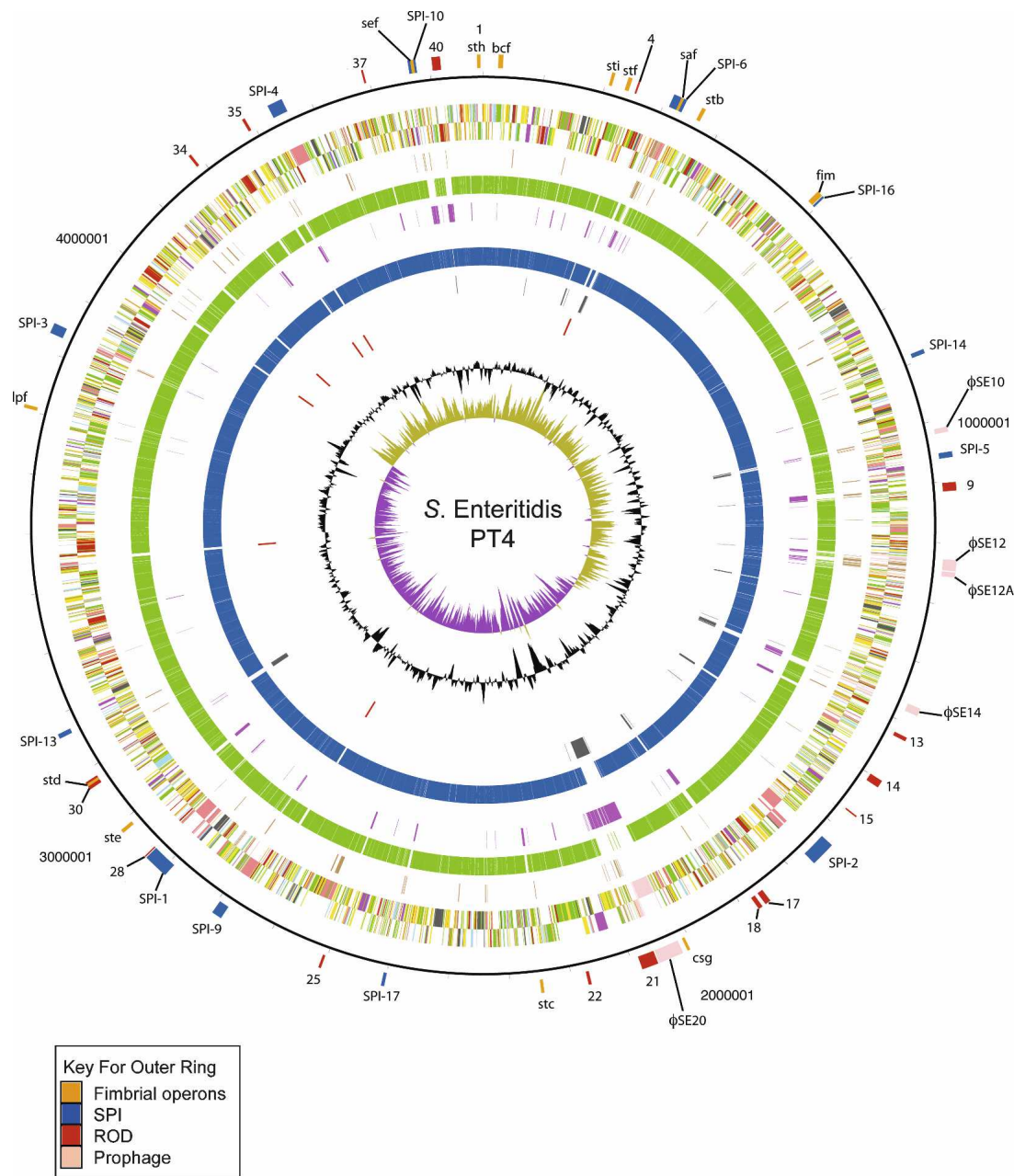
**Figure 1.** Global comparison between *S. Typhimurium*, *S. Enteritidis*, and *S. Gallinarum*. ACT comparison (<http://www.sanger.ac.uk/Software/ACT>) of amino acid matches between the complete six-frame translations (computed using TBLASTX) of the whole-genome sequences of *S. Typhimurium* LT2 (LT2), *S. Enteritidis* PT4 (PT4), and *S. Gallinarum* 287/91 (GAL). Forward and reverse strands of DNA are shown for each genome (light gray horizontal bars). The red bars between the DNA lines represent individual TBLASTX matches, with inverted matches colored blue. The position of all the fimbrial operons in these three genomes are marked as colored boxes positioned on the forward and reverse strands of DNA. Analogous fimbrial operons are colored the same. The boxes of fimbrial operons that include pseudogenes are crossed with a white line. Other genomic features are only shown if they constitute breaks in synteny between genomes. The position of the origin and terminus are marked (solid black arrows).

### Gene sets common to both *S. Enteritidis* PT4 and *S. Typhimurium* LT2

Within the core genes, there are many of the functions associated with virulence and host interactions and include SPIs and fimbrial operons. With the exception of *SPI-6*, *SPI-9*, and *SPI-10*, the other SPIs in *S. Enteritidis* PT4 are closely related to their equivalents in *S. Typhimurium* LT2 (Fig. 2) (McClelland et al. 2001). Of the three SPIs that vary, *S. Enteritidis* PT4 *SPI-10* only encodes the *sef* fimbrial operon, consistent with this region being mosaic in isolates of different serovars (Edwards et al. 2000; Collighan and Woodward 2001; Bishop et al. 2005). The *SPI-9* CDS *SE2609*, encoding a large repetitive exported protein, appears intact in *S. Enteritidis* PT4 unlike the ortholog, *STM2689*, in *S. Typhimurium* LT2. The *SPI-6* region of *S. Enteritidis* PT4 is 22 kb in size compared with 47 kb in *S. Typhimurium* LT2. *SPI-6* varies markedly in size in all the other sequenced *Salmonella*, including *S. Typhi* (McClelland et al. 2001, 2004; Parkhill et al. 2001; Chiu et al. 2005). Of the other known SPIs, *SPI-8*, *SPI-7*, and *SPI-15* are absent from *S. Enteritidis* PT4 (Parkhill et al. 2001; Vernikos and Parkhill 2006). Conversely, *SPI-17* is present in *S. Enteritidis* PT4 but absent from *S. Typhimurium* LT2. The *S. Enteritidis* PT4

*SPI-17* is a degenerate prophage encoding CDS known to be involved in O-antigen conversion in other systems (Vernikos and Parkhill 2006).

*S. Enteritidis* PT4 harbors 13 fimbrial clusters, 10 of which are highly conserved in *S. Typhimurium* LT2 with orthologous genes sharing >97% nucleotide identity and inserted at the same sites in both genomes (Fig. 1; Table 2). The only exceptions to this are *safA*, *safB*, and *stdA*, where the *S. Enteritidis* PT4 and *S. Typhimurium* LT2 orthologs show 81%, 87%, and 89% nucleotide identity, respectively. The *S. Enteritidis* PT4 fimbrial clusters not found in *S. Typhimurium* LT2 include a novel cluster we have termed *peg*, which is inserted at the same location as the *S. Typhimurium* LT2 *stc* operon and is so far restricted to *S. Enteritidis*, *S. Gallinarum* 287/91, and *S. Paratyphi* A. The *peg* fimbrial proteins show 58%–64% identity with their predicted functional equivalents in the *S. Typhimurium* LT2 *stc* cluster (Table 2). Of the remaining fimbrial clusters, *ste* is absent from *S. Typhimurium* LT2, but there is a deletion remnant of the *ste* major pilin subunit remaining at the analogous site (*S. Typhimurium* LT2; positions 3,102,016–3,102,150 bps). Fimbrial operon *stj* is present in *S. Typhimurium* LT2 and replaces a gene of unknown function still present in *S. Enteritidis* PT4 (*SEN4331A*). Thus, in common



**Figure 2.** Circular representation of the *S. Enteritidis* PT4 chromosome. From the outside in, the *outer* circle 1 marks the position of regions of difference (mentioned in the text) and is detailed in Table 2. Circle 2 shows the size in base pairs. Circles 3 and 4 show the position of CDS transcribed in a clockwise and anti-clockwise direction, respectively (for color codes see below); circle 5 shows the position of *S. Enteritidis* PT4 pseudogenes. Circles 6 and 8 show the position of *S. Enteritidis* PT4 genes that have orthologs (by reciprocal FASTA analysis) in *S. Typhimurium* strain LT2 (all CDS colored green) and *S. Gallinarum* strain 287/91 (all CDS colored blue), respectively. Circles 7 and 9 show the position of *S. Enteritidis* PT4 genes that lack orthologs (by reciprocal FASTA analysis) in *S. Typhimurium* strain LT2 (all CDS colored pink) and *S. Gallinarum* strain 287/91 (all CDS colored gray), respectively. Circle 10 shows the position of *S. Enteritidis* PT4 rRNA operons (red). Circle 11 shows a plot of G + C content (in a 10-kb window). Circle 12 shows a plot of GC skew ( $[G - C]/[G + C]$ ; in a 10-kb window). Genes in circles 3 and 4 are color-coded according to the function of their gene products: dark green, membrane or surface structures; yellow, central or intermediary metabolism; cyan, degradation of macromolecules; red, information transfer/cell division; cerise, degradation of small molecules; pale blue, regulators; salmon pink, pathogenicity or adaptation; black, energy metabolism; orange, conserved hypothetical; pale green, unknown; and brown, pseudogenes.

with other promiscuous salmonellae, *S. Enteritidis* PT4 harbors multiple functional fimbrial operons (Townsend et al. 2001).

In addition to the gene remnants found for the *ste* fimbrial operon *S. Enteritidis* PT4, RODs *ROD17*, *ROD25*, *ROD34*, *ROD35*, and *ROD37* (Table 2) that fall outside of the core

gene-set also have discernable remnants in *S. Typhimurium* LT2 or are conserved in other *S. enterica* and so are likely to have been present in a precursor of *S. Enteritidis* PT4, shared with *S. Typhimurium* LT2, and subsequently deleted from *S. Typhimurium* LT2.

### Gene sets only present in *S. Enteritidis* PT4 but not *S. Typhimurium* LT2

By analyzing the genetic context of the *S. Enteritidis* PT4 specific RODs, we distinguish between those likely to have been acquired independently from those that may have been deleted from *S. Typhimurium* LT2 (discussed above). Examples of likely acquisitions include *SPI-17*, *ROD4*, *ROD9*, *ROD13*, *ROD21*, *ROD22*, *ROD28*, *ROD40*,  $\phi$ SE14, and  $\phi$ SE20 (Table 2). RODs unique to *S. Enteritidis* PT4 include potentially mobile genomic islands, clusters of genes encoding metabolic functions and prophage-like elements, as well as a variable assortment of fimbrial operons already described (summarized in Table 2).

### Genomic islands

*ROD21* is the only *S. Enteritidis* PT4 genomic island not found in *S. Typhimurium* LT2 and has features characteristic of mobile genetic elements (Table 2). *ROD21* shares significant structural conservation with conserved genomic loci present in a range of bacteria. These islands all display an unusual G + C profile, whereby regions conserved between islands show a higher G + C content compared with the variable island-specific regions (Fig. 4) (Williamson and Free 2005; this study). Surprisingly, most of the *ROD21*-related islands encode paralogs of H-NS (*hnsB*) and/or an H-NS antagonist, *hnsT* (Williamson and Free 2005; Navarre et al. 2006; Doyle et al. 2007). These paralogs may play a role in

relieving any potential fitness burden associated with sequestering H-NS to these low G + C DNA elements (Doyle et al. 2007).

Of the other *S. Enteritidis* PT4-specific RODs, *ROD13* encodes five CDS displaying sequence similarities and synteny with genes associated with the uptake and catabolism of the hexonate sugar acid *L*-idonate encoded by the *gntII* locus of *Escherichia coli* (Table 2) (Bausch et al. 1998). Although the substrate for this system is unclear, it is known that colonic mucus contains several sugar acids that represent an important source of nutrients and that *E. coli* mutants unable to utilize them are unable to colonize the mouse large intestine (Sweeney et al. 1996). Moreover, genes involved in the transport of gluconate and related hexonates are up-regulated in *S. Typhimurium* in macrophage, suggesting that they may also be an important source of carbon for intracellular bacteria (Eriksson et al. 2003).

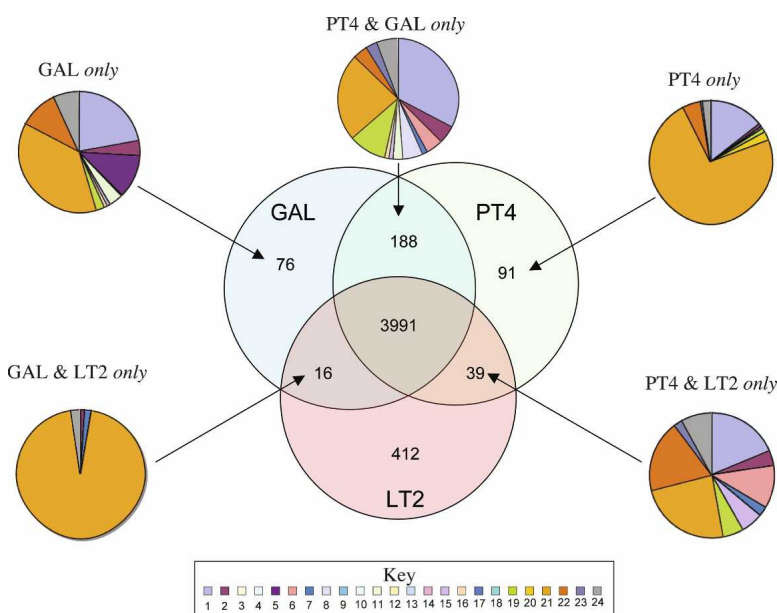
The *S. Enteritidis* PT4 RODs also include loci that are highly variable in the salmonellae and, for *ROD40*, between the wider *Enterobacteriaceae*. *ROD40* locus encodes a Type I restriction/modification system and is analogous to the variable *E. coli* immigration control region (ICR) (Raleigh 1992; Titheradge et al. 1996).

### Prophage

Prophage are known to drive diversity in *S. enterica*, and thus, it is not surprising that many *S. Enteritidis* PT4-specific RODs are prophage-like elements, including  $\phi$ SE10,  $\phi$ SE12,  $\phi$ SE12A,  $\phi$ SE14, and  $\phi$ SE20 (Fig. 2; Table 2) (Thomson et al. 2004; Cooke et al. 2007). All these prophage regions are related and carry the same cargo genes as prophage found previously in other *S. enterica* (see Table 2), including genes encoding type three secretion system (TTSS) effector proteins—*sseK3*, *sspH2*, *gogA*, *sseI*, and *sopE*; the PhoPQ-activated genes *pagK* and *pagM*; as well as *sodCI* encoding a Cu/Zn superoxide dismutase known to be an important colonization factor for *S. Typhimurium* (Stanley et al. 2000; Figueroa-Bossi et al. 2001; Mmolawa et al. 2003; Thomson et al. 2004). Of the six prophage-related regions, only  $\phi$ SE20 appears intact and probably represents a recent insertion event, whereas remnants of  $\phi$ SE12A are also present at the same location in *S. Typhimurium* LT2 and probably represent the most ancient phage insertion that has been maintained in these two *Salmonella* lineages. However, the number of remnants and intact cargo genes found on the *S. Enteritidis* PT4 prophage highlights the importance of these elements for gene sampling and increasing the overall diversity and even pathogenic potential of salmonellae.

### Whole-genome sequence of *S. Gallinarum* 287/91 and comparisons with *S. Enteritidis* PT4 and *S. Typhimurium* LT2

One of the striking features of the *S. Gallinarum* 287/91 genome is the high simi



**Figure 3.** Distribution of orthologous CDS in *S. Enteritidis* PT4, *S. Typhimurium* LT2, and *S. Gallinarum* 287/91. The Venn diagram shows the number of genes unique or shared between two other *S. enterica* serovars (see Methods). The associated pie charts show the breakdown of the functional groups assigned for CDS in relevant sections of the Venn diagram. Color code for the pie charts is as follows: (1) hypothetical proteins, (2) conserved hypothetical proteins, (3) chemotaxis and motility, (4) chromosomal replication, (5) chaperones, (6) protective responses, (7) transport and binding proteins, (8) adaptations to atypical conditions, (9) cell division, (10) macromolecule degradation, (11) synthesis and modification of macromolecules, (12) amino acid biosynthesis, (13) biosynthesis of cofactors, prosthetic groups and carriers, (14) central intermediary metabolism, (15) small-molecule degradation, (16) energy metabolism, (17) fatty acid biosynthesis, (18) nucleosides and nucleotide biosynthesis and metabolism, (19) periplasmic/exported/lipoproteins, (20) ribosomal proteins, (21) laterally acquired (including prophage CDS), (22) pathogenicity and virulence, (23) general regulation, and (24) miscellaneous function. PT4 indicates *S. Enteritidis* PT4; LT2, *S. Typhimurium* LT2; and GAL, *S. Gallinarum* 287/91

**Table 2.** The *S. Enteritidis* PT4 variable genome regions identified by genome sequencing

Label <sup>a</sup>	PT4 CDS range	GAL CDS range	Locus name(s) <sup>b</sup>	Size in PT4 <sup>c</sup>	General description of locus	Where present
bcf	SEN0020–SEN0027	SG0023–SG0030	bcf	7.6 kb	Fimbrial operon (bovine colonization factor)	GAL PT4 LT2
sti	SEN0179–SEN0182	SG0177–SG0180	sti	4.9 kb	Fimbrial operon ( <i>S. Typhi</i> I)	GAL PT4 LT2
stf	SEN0200–SEN0205	SG0199–SG0204	stf	6.4 kb	Fimbrial operon ( <i>S. Typhi</i> F)	GAL PT4 LT2
4	SEN0216	NP	ROD4	3 kb	Viral enhancing factor (metalloprotease)	PT4, NI: LT2, or GAL
SPI-6	SEN0267–SEN0290	SG0263–SG0318	SPI-6	17.6 kb (44 kb)	<i>Salmonella</i> pathogenicity island (tRNA- <i>asp</i> )	GAL PT4 LT2. Variable in all three (Parkhill et al. 2001)
saf	SEN0281–SEN0284	SG0308–SG0312	saf	4.3 kb	Part of SPI-6. Fimbriae: <i>Salmonella</i> atypical fimbriae	GAL PT4 LT2
stb	SEN0319–SEN0323	SG0346–SG0350	stb	5.9 kb	Fimbrial operon ( <i>S. Typhi</i> B)	GAL PT4 LT2
fim	SEN0524–SEN0533	SG0555–SG0564	fim	9 kb	Fimbrial operon (tRNA- <i>arg</i> )	GAL PT4 LT2
SPI-16	SEN0535–SEN0537	SG0567–SG0569	SPI-16	3.3 kb	Phage remnant. Cargo: LPS modification genes (tRNA- <i>arg</i> )	GAL PT4 LT2 (Vernikos and Parkhill 2006)
SPI-14	SEN0800–SEN0805	SG0835–SG0840	SPI-14	6.8 kb	Electron transfer and regulatory CDS	GAL PT4 LT2 (Shah et al. 2005)
φSE10	SEN0908A–SEN0921	NP	φSE10	8.2 kb	Prophage (remnant). Related to φGifsy-2. Cargo: <i>sse</i> , <i>gtgE</i> , <i>gtgF</i>	PT4 LT2, NI: GAL
SPI-5	SEN0951–SEN0958	SG0976–SG0985	SPI-5	6.6 kb	<i>Salmonella</i> pathogenicity island (tRNA- <i>ser</i> )	GAL PT4 LT2 (Wood et al. 1998)
9	SEN0995–SEN1013B	SG1023–SG1058	ROD9	13.7 kb (42 kb)	RHS element, exported proteins and an lcmF-like CDS	GAL, NI: LT2. Degenerate in PT4
φSE12	SEN1131–SEN1156	SG1180–SG1234	φSE12 [φSG12]	18 kb (45 kb)	Prophage (remnant). Related to φGifsy-2. Cargo: <i>sodC</i> , <i>ompX</i> , <i>sopE</i> , <i>gogA</i> , <i>hokW</i>	GAL, NI: LT2. Degenerate in PT4
φSE12A	SEN1158–SEN1171B	SG1236–SG1249	φSE12A [φSG12A]	8 kb	Prophage (remnant). Related to φGifsy-2. Cargo: <i>pagK</i> , <i>pagM</i>	GAL PT4 LT2
φSE14	SEN1378–SEN1398	NP	φSE14	12.6 kb	Prophage (remnant). Related to <i>S. Typhi</i> φST18	PT4, NI: LT2 GAL
13	SEN1432–SEN1436	SG1499–SG1503	ROD13	6 kb	Possible hexonate uptake and catabolism operon	GAL PT4, NI LT2
14	SEN1499–SEN1509	SG1573*–SG1576*	ROD14	11.7 kb (1.8 kb)	Drug efflux system, <i>pqxA</i>	GAL PT4 LT2. Variable in all three
15	SEN1565–SEN1567	SG1636*	ROD15	2.2 kb (0.6 kb)	Exported protease, <i>mdtI</i> and <i>mdtJ</i> (Nal <sup>r</sup> , Fos <sup>r</sup> , Det <sup>r</sup> )	PT4 LT2. Remnant in GAL
SPI-2	SEN1623–SEN1666	SG1694–SG1738	SPI-2	39.8 kb	(Nishino and Yamaguchi 2001) <i>Salmonella</i> pathogenicity island (tRNA- <i>val</i> )	PT4 GAL LT2 (Hensel 2000)
17	SEN1751–SEN1758	SG1824–SG1832	ROD17	9.3 kb	Putative membrane-transport system	PT4 GAL. Remnant in LT2 (1,370,980–1,371,012)
18	SEN1766–SEN1769	SG1841*	ROD18	6.9 kb (0.3 kb)	<i>nipA</i> –outer-membrane scaffold	PT4 LT2. Remnant in GAL
csg	SEN1903–SEN1909	SG1977–SG1983	csg	4.4 kb	Curli fimbrial operon	PT4 GAL LT2
φSE20	SEN1919A–SEN1966	NP	φSE20	40.6 kb	Prophage. Related to φST64B. Cargo: <i>sopE</i> , <i>sppH1</i> , <i>orgA</i> (tRNA- <i>ser</i> )	PT4, NI: GAL LT2
21	SEN1970–SEN1999	SG1996–SG2025	ROD21	26.5 kb	Genomic island (61 bp DR; tRNA- <i>asn</i> ; 37.5% G + C) Cargo: <i>hnsT</i> , <i>hnsB</i>	PT4 GAL, NI: LT2
22	SEN2085A–SEN2085D	SG2117–SG2120	ROD22	4.9 kb	Group D LPS O-chain genes <i>rfbE</i> and <i>rfbS</i>	PT4 GAL. Different genes in LT2 (group B O-chain)
peg	SEN2144A–SEN2145B	SG2182–SG2186	peg	4.8 kb	Fimbrial operon	PT4 GAL, NI: LT2
SPI-17	SEN2375A–SEN2380A	SG2425–SG2430A	SPI-17	3.6 kb	Prophage remnant. Cargo: <i>gtrA</i> , <i>gtrB</i> , <i>gtrC</i> (tRNA- <i>arg</i> )	PT4 GAL, NI: LT2 (Vernikos and Parkhill 2006)
25	SEN2471–SEN2473	SG2522–SG2524	ROD25	4 kb	Membrane transport system	PT4 GAL. Remnant in LT2 (STM2492*)
SPI-9	SEN2609–SEN2612	SG2666–SG2671	SPI-9	16.3 kb	<i>Salmonella</i> pathogenicity island (10Sa_RNA)	PT4 GAL LT2 (Parkhill et al. 2001)
SPI-1	SEN2703–SEN2744	SG2764–SG2806	SPI-1	40.2 kb	<i>Salmonella</i> pathogenicity island	PT4 GAL LT2 (Hansen-Wester and Hensel 2001)
28	SEN2746–SEN2746A	SG2808–SG2811	ROD28	2 kb	Membrane proteins	PT4 GAL, NI: LT2

(continued)

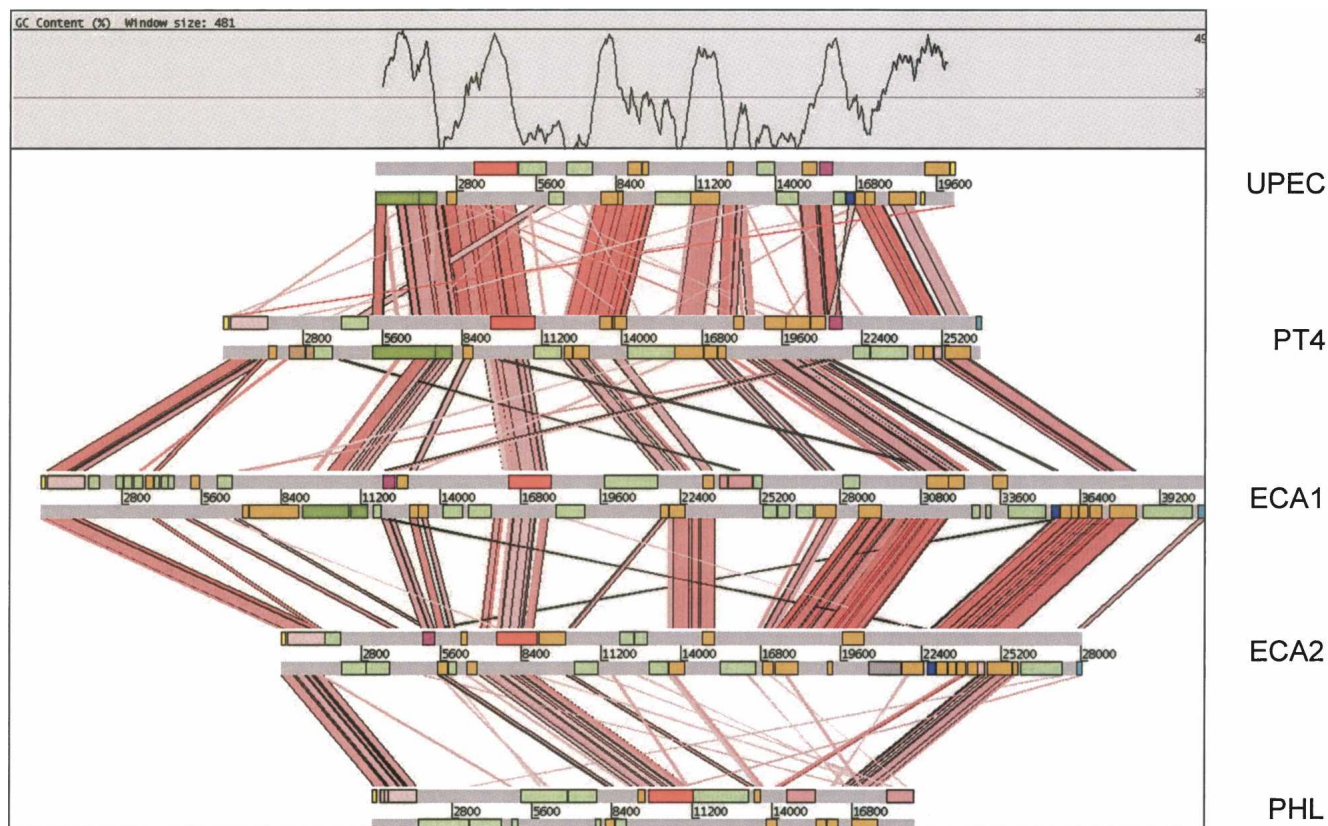
**Table 2. Continued**

Label <sup>a</sup>	PT4 CDS range	GAL CDS range	Locus name(s) <sup>b</sup>	Size in PT4 <sup>c</sup>	General description of locus	Where present
ste	SEN2794–SEN2799	SG2859–SG2864	ste	5.6 kb	Fimbrial operon ( <i>S. Typhi</i> E)	PT4 GAL. Remnant in LT2 (major pilin remnant: 3,102,016–3,102,150)
30	SEN2864–SEN2878	SG2930*	ROD30	13 kb (0.16 kb)	<i>rnaA</i> , the <i>std</i> fimbrial, operon	PT4 LT2. Remnant in GAL
std	SEN2871–SEN2873	NP	<i>std</i>	5.0 kb	Within <i>ROD30</i> . Fimbrial operon ( <i>S. Typhi</i> D)	PT4 LT2. Ni: GAL
SPI-13	SEN2960–SEN2966	SG3011–SG3017	SPI-13	7.4 kb	<i>Salmonella</i> pathogenicity island (tRNA- <i>phe</i> )	PT4 GAL LT2 (Shah et al. 2005)
lpf	SEN3459–SEN3463	SG3793–SG3798	<i>lpf</i>	5.5 kb	Fimbrial operon (long polar fimbriae)	PT4 GAL LT2
SPI-3	SEN3572–SEN3586	SG3665–SG3680	SPI-3	16.6 kb	<i>Salmonella</i> pathogenicity island (tRNA- <i>sef</i> )	PT4 GAL LT2 (Blanc-Potard et al. 1999)
34	SEN3896–SEN3898	SG3312–SG3314	ROD34	4.2 kb	Amino acid metabolic CDS	PT4 GAL, Ni: LT2
35	SEN3978–SEN3981	SG4054–SG4058	ROD35	4.5 kb	Unknown	PT4 GAL, Ni: LT2
SPI-4	SEN4026–SEN4032	SG4100–SG4105	SPI-4	25 kb	<i>Salmonella</i> pathogenicity island	PT4 GAL LT2 (Wong et al. 1998; Parkhill et al. 2001)
37	SEN4165–SEN4166	SG4241–SG4242	ROD37	3 kb	Unknown	PT4 GAL, Ni: LT2
SPI-10	SEN4244–SEN4254	SG4311–SG4325	SPI-10	10 kb	<i>Salmonella</i> pathogenicity island (tRNA- <i>leu</i> )	GAL PT4, Ni: LT2 (Parkhill et al. 2001)
sef	SEN4247–SEN4251	SG4318–SG4322	<i>sef</i>	5.1 kb	Fimbrial operon ( <i>Salmonella</i> Enteritidis fimbriae)	PT4 GAL, Ni: LT2
40	SEN4283–SEN4292	SG4349–SG4357	ROD40	13.5 kb (10 kb)	Type I restriction-modification system	PT4. Degenerate in GAL, Ni: LT2
sth	SEN4347–SEN4351	SG4413–SG4417	<i>sth</i>	5.5 kb	Fimbrial operon ( <i>S. Typhi</i> H)	PT4 GAL LT2
42	SEN3843*–SEN3844*	SG3367–SG3368	ROD42	1 kb	C4-dicarboxylate transporters	GAL LT2. Deleted from PT4

<sup>a</sup>Labels used to mark these regions on the outer ring of Figures 2 and 5.

<sup>b</sup>Square brackets indicate the name in *S. Gallinarum* 287/91.

<sup>c</sup>Numbers in parentheses indicate the size of the analogous region in *S. Gallinarum*. Only shown when significantly different from that found in *S. Enteritidis* PT4. (\*): Gene remnant; (DR) direct repeat; (NI) not detected in; (LPS) lipopolysaccharide locus; (LT2) *S. Typhimurium* LT2; (GAL) *S. Gallinarum* 287/91; (PT4) *S. Enteritidis* PT4; (Nal<sup>r</sup>) nalidixic acid resistance; (Fos<sup>r</sup>) fosfomycin resistance; (Det<sup>r</sup>) detergent resistance.



**Figure 4.** Comparison of the *ROD21* locus of *S. Enteritidis* with related genomic islands. ACT comparison (<http://www.sanger.ac.uk/Software/ACT>) of amino acid matches between the complete six-frame translations (computed using TBLASTX) of *ROD21* compared with related loci uropathogenic *E. coli* strain CFT073 (UPEC), *Erwinia carotovora* sbsp. *atroseptica* strain SCRI1043 (two loci: *ECA1* and *ECA2*), and *Photobacterium luminescens* sbsp. *laumondii* TT01 (PHL; see Methods). The red bars spanning between the genomes represent individual TBLASTX matches. CDS are marked as colored boxes positioned on the horizontal gray DNA bars: (orange) genes conserved in two or more of the genomic islands; (light green) variable genes of unknown function; (dark pink) *hnsB*; (dark blue) *hnsT*; (light pink) integrase; (dark green) type IV pilin-associated genes; (red) plasmid-related mobility functions; (salmon pink) transposase-related genes; (yellow) tRNA genes; (light blue) repeats. The G + C profile for the UPEC loci is shown above. The scale is marked in base pairs.

ilarity with *S. Enteritidis* PT4 compared with *S. Typhimurium* LT2 (Figs. 3, 5). The average nucleotide identities of orthologs shared between *S. Gallinarum* 287/91 and *S. Enteritidis* PT4 were higher (99.7%) than those found in LT2 (98.93%). Another obvious feature is the massive accumulation of pseudogenes in *S. Gallinarum* 287/91 compared with *S. Enteritidis* PT4 and *S. Typhimurium* LT2. The genome of *S. Gallinarum* 287/91 is slightly smaller than *S. Enteritidis* PT4, carries significantly fewer tRNA genes (Table 1), and is colinear except for a single inversion (817 kb; about the rRNA operons) and translocation of a region (49 kb) located between two different rRNA operons (Figs. 1, 5).

The number of CDS unique to *S. Gallinarum* 287/91 (76) or shared exclusively between *S. Gallinarum* 287/91 and *S. Typhimurium* LT2 (16) was small and predominantly phage-associated (Figs. 3, 5). Moreover, genes from both of these categories all fell within regions (*SPI-6*, *ROD9*, *ROD42*, and  $\phi$ SG12) that are present in *S. Enteritidis* PT4 but appear to be in the process of being lost. Consequently, these genes are unlikely to be recent acquisitions by *S. Gallinarum* 287/91.

Of the 130 CDS specific to *S. Enteritidis* PT4, compared with *S. Gallinarum* 287/91, those associated with *ROD4* and prophages  $\phi$ SE10,  $\phi$ SE14, and  $\phi$ SE20 (82 CDS) appear to be recent acquisitions with no evidence of them ever being present in

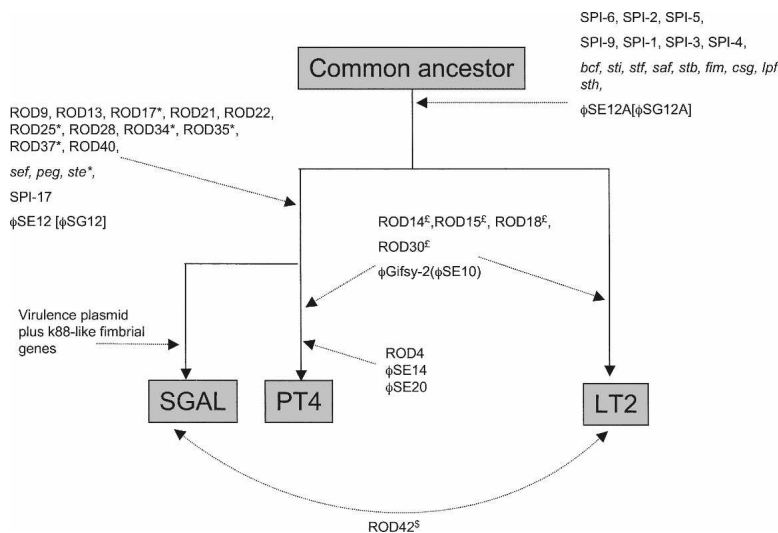
*S. Gallinarum* 287/91 (Fig. 3; Table 2). Of the remaining 48 CDS in this category, 21 were located on loci (*ROD15*, *ROD18*, and *ROD30*) (Table 2) almost entirely deleted from *S. Gallinarum* 287/91. The others were located on shared loci such as *ROD14* and *SPI-6* that are degenerate in both serotypes, compared with *S. Typhimurium* LT2. The functions that these RODs encode in *S. Enteritidis* PT4 are summarized in Table 2.

Thus, we provide compelling genetic evidence that *S. Enteritidis* and *S. Gallinarum* are recently diverged clones. On this conclusion, we have plotted the most parsimonious explanation for the observed gene flux following the divergence of *S. Typhimurium* LT2, *S. Enteritidis* PT4, and *S. Gallinarum* 287/91 (Fig. 6).

#### Functional gene loss and pseudogene formation

In addition to the large scale deletion, there is further evidence of reductive evolution in *S. Gallinarum* 287/91 in the form of 309 putative pseudogenes that carry frameshifts or premature stop codons or that are remnants of genes present in other bacteria. Remarkably, this represents ~7% of the total coding capacity of the genome and includes genes from many functional categories, including metabolism and virulence (for a full list, see Supplemental Table 1).





**Figure 6.** Line diagram to represent the whole-genome differences of *S. Enteritidis* PT4, *S. Typhimurium* strain LT2, and *S. Gallinarum* strain 287/91. A summary of the observed loss and gain of RODs described in Table 2. The diagram is based on the assumption that following the divergence of PT4 and GAL have subsequently diverged. Branches are not intended to infer phylogenetic distance. Evidence that a locus was once present in LT2, PT4, or GAL (see legend to Figure 1) but has subsequently been deleted from that genome is marked by the suffix \*, \$, or £, respectively. Brackets indicate the name for locus in GAL. Parentheses indicate the name for locus in PT4. Dotted arrows mark the position in the pseudo-tree at which that ROD(s) appears

these three pathways was probably the precursor to the degeneration of the others in *S. Gallinarum* 287/91. Mutations within the *cbi*, *pdu*, and *ttr* genes was also a feature of the *S. Typhi* CT18 genome (Table 3), suggesting that these mutations may be characteristic of more invasive *Salmonella* serotypes.

Uniquely among the *Salmonella*, *S. Gallinarum* 287/91 and *S. Pullorum* are unable to make glycogen (Supplemental Table 2) (McMeehan et al. 2005). The genome sequence data revealed that mutations in the glycogen biosynthetic pathway are extensive in *S. Gallinarum* 287/91, including *glgA*, *glgB*, and *glgC*, which are responsible for all steps in biosynthesis. Although the significance of these mutations are not clear, they may explain in part the poor survival of this bacterium outside of the host (McMeehan et al. 2005).

Several of the identified *S. Gallinarum* 287/91 pseudogenes lie in amino acid catabolic or biosynthetic pathways. *Salmonella* encodes three pathways for arginine degradation (Reitzer 2003), and *S. Gallinarum* 287/91 carries mutations in two of these: There are deletions or frameshifts in the genes of the arginine *N*-succinyltransferase (AST) (*astA*) and arginine deiminase (ADI) (*arcA*) pathways, respectively. *S. Gallinarum* 287/91 also carries a mutation in *speC* encoding the ornithine decarboxylase, making the one remaining intact arginine catabolic pathway, arginine decarboxylase pathway (ADC), an essential biosynthetic route for putrescine. The mutation in *speC* is also likely to explain the inability of *S. Gallinarum* to decarboxylate ornithine, a defining feature of this *Salmonella* serovar (Supplemental Table 2) (Crichton and Old 1990).

### Pseudogenes potentially involved in virulence and host adaptation

*S. Gallinarum* 287/91 is defined as being nonmotile. *S. Gallinarum* 287/91 carries 50 genes associated with motility and chemotaxis, distributed over three loci. Of these, five genes, present

in two loci, carry mutations that explain the nonmotile phenotype, including *cheM*, *flhA*, *flhB*, *flgK*, and *flgI* (Supplemental Table 1).

Of the 13 fimbrial operons detected in *S. Enteritidis* PT4, the *std* fimbrial operon is not present in *S. Gallinarum* 287/91 (see *ROD30* above). The remaining 12 *S. Gallinarum* 287/91 fimbrial operons are identical to those in *S. Enteritidis* PT4 except for the mutations in genes within operons *sti*, *stf*, *saf*, *stb*, *peg*, *lpf*, *sef*, and *sth* (see Supplemental Table 1). The level of pseudogene formation within these 12 fimbrial gene clusters (16%) is over twice that of the genome average (7%). Only operons *fim*, *bcf*, *csg*, and *ste* remain undisrupted on the *S. Gallinarum* 287/91 chromosome (summarized in Fig. 1). However, both *S. Enteritidis* and *S. Gallinarum* carry fimbrial operons on their virulence plasmids. The *S. Enteritidis* virulence plasmid carries five genes—*pefA*, *pefB*, *pefC*, *pefD*, and *pefR*—highly conserved with those of *S. Typhimurium* LT2 (Woodward and Kirwan 1996). The *pef* operon is not present on the *S. Gallinarum* 287/91 plasmid; in its place are

three intact fimbrial genes displaying sequence similarity with those of the *E. coli* K88 fimbrial gene cluster (Rychlik et al. 1998). This fimbrial operon represents the only evidence of *S. Gallinarum* 287/91 having acquired new functions since the split from *S. Enteritidis*. It would be interesting to know if any isolates of *S. Enteritidis* carry such a fimbrial operon and whether this is a common characteristic of all *S. Gallinarum* strains. Significantly, the host-adapted *S. Typhi* harbors novel fimbrial genes, including Type IV pili associated with the acquisition of mobile elements (Pickard et al. 2003), and like *S. Gallinarum* 287/91, there is an elevated level of mutation in fimbrial genes (14% compared with the genome average of 4.4%), again suggesting parallel paths toward host adaptation (Table 3).

*Salmonella* can express several paralogous TTSS effector proteins, which show a degree of functional redundancy, for example, *sopE* and *sopE2* (Friebel et al. 2001). Other effectors related by sequence include *pipB* and *pipB2*, and *sifA* and *sifB*. *S. Gallinarum* 287/91 has lost one of each of these paralogous pairs. Other *S. Gallinarum* 287/91 TTSS effector genes that carry mutations include *sopA*, which has been implicated in *S. Typhimurium*-induced intestinal inflammation (Zhang et al. 2006). Using an antibody against the C-terminal portion of SopA, we detected a secreted protein of the expected size in *S. Enteritidis* PT4 but not *S. Gallinarum* 287/91 (data not shown), consistent with the location of a stop codon prior to the mAb-binding region in *S. Gallinarum* SopA predicted by the genome sequence (Supplemental Table 2). SopA influences *Salmonella*-induced enteritis, and taken together with the attrition of other Type III secreted effectors, this may partially dictate the differential virulence of the serovars in mammalian hosts.

As well as additional pseudogenes associated with cell interactions, again like *S. Typhi*, *S. Gallinarum* 287/91 carries mutations in genes also associated with shedding (*shdA* and *ratB*), drug resistance, DNA restriction/modification, and protective re-

**Table 3.** Summary of the common traits identified among the functions of genes lost independently by *S. Typhi* CT18 and *S. Gallinarum* 287/91

Process/pathway	<i>S. Gallinarum</i> 278/91 <sup>a</sup>	<i>S. Typhi</i> CT18 <sup>b</sup>
Cell interactions	<i>slrP, sopA, sifB, sspH2, sinH, pipB2, pagK, bigA</i>	<i>sopD2 (STY0971), sopA (STY2275), sopE2 (STY1987), sseJ (STY1439a), cigR (STY4024), misL (STY4030), marT (STY4027), sivH (STY2767), slrP (STY0833), bigA (STY4318)</i>
Fecal shedding	<i>shdA, ratB</i>	<i>shdA (STY2755), ratB (STY2758), sivH (STY2767)</i>
Fimbriae	<i>stdΔ, stiC, stff, safC, stbC, pegC, lpfC, sefD, sefC, sthB, sthA, sthE</i>	<i>bcfC (STY0026), fimI (STY0590), steA (STY3084), safE (STY0333), stgC (STY3920), ushA (STY0539), sefA (STY4836a), sefD (STY4839), sefR (STY4841), sthC (STY4938), sthE (STY4938)</i>
Flagella/motility	<i>cheM, flhB, flhA, flgK, flgI</i>	<i>fliB (STY2166)</i>
Type I restriction modification	<i>hsdR, hsdM</i>	<i>hsdM (STY4833)</i>
Type III restriction modification		
Restriction enzyme StyLT1	<i>mod</i>	<i>res (STY0389)</i>
Cobalamin biosynthesis	<i>pocR, cobD, cbiD, cbiC, cbiO</i>	<i>cbiM (STY2226), cbiK (STY2229), cbjI (STY2231)</i>
Propanediol utilization	<i>pduG, pduO</i>	<i>pduN (STY2254)</i>
Metal/drug resistance and transport		
Copper	<i>cusA, cusS</i>	<i>cusA (STY0610), cusS (STY0609a)</i>
Nickel/cobalt	<i>rcnA, cusA, cusS</i>	<i>rcnA (STY3169)</i>
Nickel	<i>nxiA, ArcnA</i>	<i>nxiA (STY2901)</i>
Acriflavin	<i>acrF, nxiA</i>	<i>acrE (STY3569), acrF (STY3570)</i>
Tetrathionate respiration	<i>ttrB, ttrC</i>	<i>ttrS (STY1735)</i>
Trehalose degradation/synthesis	<i>treC</i>	<i>treA (STY1924)</i>
Hydrogenase I	<i>hyaF</i>	<i>hyaB2 (STY1525), hyaA (STY1319)</i>
Ornithine catabolism	<i>speC</i>	<i>speC (STY3270), speF (STY0739)</i>
Amino acid catabolism		
L-serine/L-threonine	<i>tdcG</i>	<i>tdcC (STY3426)</i>
Cellulose biosynthesis	<i>bcsG</i>	<i>bcsC (STY4184)</i>
Surface polysaccharide		
LPS O-chain	<i>gtrB</i>	<i>gtrB (STY2627b)</i>
LPS core	<i>rfaZ (waaZ)</i>	<i>wcaK (STY2311), wcaD (STY2324), wcaA (STY2328)</i>
Alternative terminal electron acceptors		
Dimethyl sulfoxide reductase	<i>dmsA2, dmsA1</i>	<i>dmsA2 (STY4503), dmsB2 (STY4506)</i>
Trimethylamine N-oxide (TMAO)	<i>torS</i>	<i>torR (STY3954), torC (STY3955)</i>
Carbon source		
D-Glucarate uptake and degradation	<i>gudD</i>	<i>gudP (STY4097)</i>
Maltodextrin and Maltose associated	<i>malS, malY, malX</i>	<i>malY (STY1657a), malX (STY1657)</i>

<sup>a</sup>For systematic gene identifiers and a description of function, see Supplemental Table 1.

<sup>b</sup>Parkhill et al. 2001.

sponses (Supplemental Table 1). The majority of *S. Enteritidis* isolates can produce a biofilm, of which cellulose is a key component. While mutations in biofilm production may not measurably affect virulence, they are significantly less resistant to chemical and mechanical stress. Consequently, this is likely to be an adaptation by *Salmonella* to survival in the environment but has also been suggested to prolong retention in the gut (Solano et al. 2002). We have shown experimentally that *S. Gallinarum* 287/91 is unable to make cellulose, and this is likely to be explained by a mutation in *bcsG* (Supplemental Table 2). This is consistent with the reduced ability of *S. Gallinarum* to colonize the gut compared with *S. Enteritidis*.

## Conclusions

The data presented in this report provide several clear messages, some of which may be experimentally tractable. Comparative analysis of the genomes of *S. Enteritidis* PT4 and *S. Gallinarum* 287/91 shows that representative strains of these two *S. enterica* serovars are highly related and that *S. Gallinarum* may be a direct descendant of *S. Enteritidis*. Importantly, *S. Enteritidis* is promiscuous, being able to colonize and infect multiple hosts, including chickens, cattle, mice, and humans, in addition to producing

murine typhoid. Whereas *S. Gallinarum* is highly restricted to causing a typhoid-like disease in avian species, it is relatively noninfectious in other hosts, including mice, and does not colonize the gut of animals. Thus, we suggest that there is an experimental opportunity to use genetic approaches to define the genetic basis of host restriction by directly comparing the pathogenicity of strains of *S. Enteritidis* and *S. Gallinarum* in murine and chicken models.

Previous genome analyses on host-restricted salmonellae has involved human-restricted serovars, including *S. Typhi* and *S. Paratyphi*, limiting experimental tractability. Nevertheless genome comparisons of host-restricted/adapted *S. enterica* serovars, and indeed of other pathogens, indicate that loss of gene function may be a common evolutionary mechanism through which host adaptation occurs. Gene loss not only may limit the inter-host promiscuity of the pathogen but also is likely to restrict the potential pathogenicity in the host to a more limited set of interactions. We hypothesize that gene loss may be a mechanism of targeting the invading pathogen preferentially to particular tissues or host cells and avoiding the potential stimulation of non-specific inflammation. An example here would be the loss of flagella or fimbriae, which can mediate attachment and invasion of cell surfaces and may activate pattern recognition molecules.

In addition, gene loss can influence the ability of the pathogen to survive in the external environment or even in stressful situations within the host. Table 3 provides a list of some of the common traits identified among the functions of genes lost independently by *S. Typhi* and *S. Gallinarum*. Some of the overlaps are striking, including the loss of common TTSS effectors and genes involved in common metabolic processes such as cobalamin and propanediol utilization, tetrathionate respiration, sugar uptake and utilization, hydrogenase activity, cellulose production, ornithine decarboxylase activity, and electron transport acceptor function. Some of these common traits have also been noted to have changed in representatives of gut adapted (*Y. enterocolitica*) versus systemic (*Y. pestis*) yersiniae, and again in this system, gene loss may be involved in the adaptation from a gut to a systemic lifestyle. We believe that further studies analyzing the contribution of pseudogenes and their functional alleles to host adaptation and tissue specificity and, in particular, the parallel but overlapping degradative evolutionary pathways followed by different organisms adapting to different hosts will lead to significant understanding of the mechanisms of host adaptation and host restriction and could be applicable to the less tractable human-adapted organisms, such as *S. Typhi*.

## Methods

### Bacterial strains

*S. Gallinarum* strain 287/91 was isolated from an outbreak of fowl typhoid in brown egg-laying hens by A. Berchieri, University of Sao Paulo, Jaboticabal, Brazil. It is highly virulent (>90% mortality) in susceptible breeds of chickens (P. Barrow and A. Berchier, unpubl.). It was chosen in preference to the well-characterized strain 9 (Smith 1955) because of the length of laboratory passage of the latter strain. *S. Enteritidis* phage type 4 (PT4) strain P125109 was isolated from an outbreak of human food-poisoning in the United Kingdom that was traced back to a poultry farm. The strain is highly virulent in newly hatched chickens and is also invasive in laying hens, resulting in egg contamination (Barrow 1991; Barrow and Lovell 1991). Biochemical tests for carbohydrate catabolism were performed using api 50 CH according to manufacturer's instructions (BioMerieux).

### Growth and sequencing of *S. Enteritidis* PT4 and *S. Gallinarum* 287/91

Methods for sequencing *S. Enteritidis* PT4 and *S. Gallinarum* 287/91 were identical unless stated. A single bacterial colony was picked from Congo Red agar and grown overnight in BAB broth with shaking at 37°C. Cells were collected, and total DNA (10 mg) was isolated using proteinase K treatment followed by phenol extraction. The DNA was fragmented by sonication, and several libraries were generated in pUC18 using size fractions ranging from 1.0–2.5 kb.

The whole genome sequenced to a depth of 9× coverage from M13mp18 (insert size 1.4–2 kb) and pUC18 (insert size 2.2–4.2 kb) small insert libraries using dye terminator chemistry on ABI3700 automated sequencers. End sequences from larger insert plasmid (pBACe3.6, 12–30 kb insert size) libraries were used as a scaffold.

The sequence was assembled, finished, and annotated as described previously (Parkhill et al. 2000), using the program Artemis (Berriman and Rutherford 2003) to collate data and facilitate annotation.

The genomes have been submitted to EMBL under the fol-

lowing accession numbers: *S. Enteritidis* PT4 genome, AM933172; *S. Gallinarum* 287/91 genome, AM933173.

### In silico genome analysis

The genome sequences of *S. Typhimurium* strain LT2 (McClelland et al. 2001), *S. Enteritidis* PT4, and *S. Gallinarum* 287/91 were compared pairwise using the Artemis Comparison Tool (ACT) (Carver et al. 2005). Subsequences taken from the genomes of uropathogenic *E. coli* strain CFT073 (Welch et al. 2002), *Erwinia carotovora* sbsp. *atroseptica* strain SCRI1043 (Bell et al. 2004), and *Photobacterium luminescens* sbsp. *laumondii* TT01 (Duchaud et al. 2003) were compared with ACT as above and used to construct Figure 4.

Pseudogenes had one or more mutations that would ablate expression; each of the inactivating mutations was confirmed by subsequently rechecking the original sequencing data and where necessary were resequenced.

Orthologous gene sets were identified by reciprocal FASTA searches. Only those pairs of homologous CDS were retained for further analysis where the predicted amino acid identity was ≥40% over 80% of the protein length. These genes were then subject to manual curation using gene synteny to increase the accuracy of this analysis. This strategy was applied to pairwise comparisons of the genomes of *S. Typhimurium* strain LT2, *S. Enteritidis* PT4, and *S. Gallinarum* 287/91.

### Cellulose production assay

For preparation and use of Calcofluor plates, Calcofluor white stain was obtained from Sigma as a 0.1% w/v solution. This was added to L-agar at a final concentration of 200 µg/mL as recommended by Solano et al. (2002). Bacterial cultures were inoculated and then left at room temperature for 48 h.

Colony fluorescence was examined by holding the plate over a 366-nm UV transilluminator. Controls used included *E. coli* C600 (negative control) and *S. typhimurium* SL1344 (positive control). Colony fluorescence was scored quantitatively using the controls as standards.

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