JOURNAL OF BACTERIOLOGY, May 2002, p. 2626–2633 Vol. 184, No. 10
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.10.2626–2633.2002

The Evolving Genome of *Salmonella enterica* Serovar Pullorum

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Received 20 December 2001/Accepted 15 February 2002

***Salmonella enterica* serovar Pullorum is a fowl-adapted bacterial pathogen that causes dysentery (pullorum disease). Host adaptation and special pathogenesis make *S. enterica* serovar Pullorum an exceptionally good system for studies of bacterial evolution and speciation, especially regarding pathogen-host interactions and the acquisition of pathogenicity. We constructed a genome map of *S. enterica* serovar Pullorum RKS5078, using I-*Ceu*I, *Xba*I, *Avr*II, and *Spe*I and Tn*10* insertions. Pulsed-field gel electrophoresis was employed to separate the large DNA fragments generated by the endonucleases. The genome is 4,930 kb, which is similar to most salmonellas . However, the genome of *S. enterica* serovar Pullorum RKS5078 is organized very differently from the majority of salmonellas, with three major inversions and one translocation. This extraordinary genome structure was seen in most *S. enterica* serovar Pullorum strains examined, with different structures in a minority of *S. enterica* serovar Pullorum strains. We describe the coexistence of different genome structures among the same bacteria as genomic plasticity. Through comparisons with *S. enterica* serovar Typhimurium, we resolved seven putative insertions and eight deletions ranging in size from 12 to 157 kb. The genomic plasticity seen among *S. enterica* serovar Pullorum strains supported our hypothesis about its association with bacterial evolution: a large genomic insertion (157 kb in this case) disrupted the genomic balance, and rebalancing by independent recombination events in individual lineages resulted in diverse genome structures. As far as the structural plasticity exists, the *S. enterica* serovar Pullorum genome will continue evolving to reach a further streamlined and balanced structure.**

The bacterial genus *Salmonella* consists of more than 2,400 documented species, many of which are important pathogens in humans or animals (4, 14, 16, 41, 42, 44, 45). Selander and colleagues have assembled three sets of reference strains for representative *Salmonella* species (5, 7, 8). The *Salmonella* species are very closely related to one another, as judged by genomic DNA reassociation rates (9, 15), which could be higher than 90%. Despite such close relatedness, different *Salmonella* species may have drastically different biological properties, especially in host range and the nature of diseases they cause. For example, *S. enterica* serovar Typhimurium infects many host species, including humans, mice, and fowl, but *S. enterica* serovar Typhi, a close relative of *S. enterica* serovar Typhimurium , is strictly limited to humans. *S. enterica* serovar Pullorum and *S. enterica* serovar Gallinarum, on the other hand, are both specific to fowl, but they cause distinct diseases, with *S. enterica* serovar Pullorum causing dysentery (pullorum disease) and *S. enterica* serovar Gallinarum causing typhoid fever (45, 46). Our long-term goal is, through genomic comparisons among representative *Salmonella* species, to explore the mechanisms of genomic divergence and evolution that have brought about the genetic differences and made each of the *Salmonella* species unique.

Representative strains of two *Salmonella* serovars have been completely sequenced, *S. enterica* serovar Typhimurium LT2 (33) and *S. enterica* serovar Typhi CT18 (39). Base-to-base comparisons of these sequenced *Salmonella* genomes would surely resolve all the genetic differences between them and provide new insights into the mechanisms of phylogenetic divergence and evolution of these bacteria. However, there are more than 2,400 *Salmonella* serovars, each of which is unique. Sequencing all of them is out of the question. Based on the great genetic similarity among all *Salmonella* species, we suggest and are testing a complementary approach to comparative genomics of salmonellas: determining the overall genome structure and locating genomic differences by physical mapping. This can be done on a very large number of selected *Salmonella* species within a relatively short time. Once insertions are located, they can be further analyzed by sequencing and functional studies. Deletions can be defined through comparisons with the genomic sequence of *S. enterica* serovar Typhimurium. Inversions and translocations can be very efficiently resolved by physical mapping. Previously, we have mapped large-scale genomic insertions, inversions, and translocations in a number of *Salmonella* species (18, 20, 22–24). We have now optimized the techniques to greater accuracy and higher efficiency for systematic comparisons among *Salmonella* spp.

S. enterica serovar Pullorum is highly adapted to fowl, although *S. enterica* serovar Pullorum infections in primates have been reported (36). However, because of the high specificity of *S. enterica* serovar Pullorum for fowl, *S. enterica* serovar Pullorum infections in mammals are extremely rare and therefore have not been a serious public health issue.

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As a fowl-specific pathogen, on the other hand, *S. enterica* serovar Pullorum continues to cause economic losses worldwide. The increasing problems of antibiotic resistance, long persistence of the bacteria in chickens after infection (49), and poor knowledge of the immunology of *Salmonella* infection in poultry (50) call for a better understanding of the genetics of *S. enterica* serovar Pullorum and pathogenesis of *S. enterica* serovar Pullorum infection in order to develop alternative measures for the control of this pathogen. As the first step toward this, we constructed a genome map of *S. enterica* serovar Pullorum on strain RKS5078. We located numerous insertions and deletions, ranging from 12 to 157 kb, as well as several major genomic rearrangements on the genome. These findings suggest possible roles of these genomic changes in the phylogenetic divergence and evolution of *S. enterica* serovar Pullorum and lead us to conclude that the genome of *S. enterica* serovar Pullorum is still evolving to reestablish a genomic balance and eventually to complete the process of speciation.

MATERIALS AND METHODS

Bacterial strains and cultivation conditions. *S. enterica* serovar Pullorum strains RKS5078, maintained at the *Salmonella* Genetic Stock Center (SGSC, www.ucalgary.ca/≈kesander) as SGSC2294, and RKS2242 (SGSC2295) were obtained from R. K. Selander, and strains 490 (SGSC2737), 498 (SGSC2738), 499 (SGSC2739), 501 (SGSC2740), 504 (SGSC2741), 505 (SGSC2742), 507 (SGSC2743), 509 (SGSC2745), 510 (SGSC2746), 512 (SGSC2747), 513 (SGSC2748), 514 (SGSC2749), 515 (SGSC2750), and R278 (SGSC2751) were obtained from C. Poppe. Previously, we have made I-*Ceu*I maps on two *S. enterica* serovar Pullorum strains, RKS2266 (SGSC2508) and RKS2246 (SGSC2509) (28), so these two strains were not included in this study. RKS2266 and RKS2246, however, will be compared with RKS5078 in this study.

All *S. enterica* serovar Pullorum strains were identified serologically (1, 9,12:—:—) and distinguished from *S. enterica* serovar Gallinarum (also 1,9,12:—:—) by the ornithine test (*S. enterica* serovar Pullorum produces rapid decarboxylation of ornithine, whereas *S. enterica* serovar Gallinarum does not). Tn*10* insertion mutants of *S. enterica* serovar Typhimurium LT2 were obtained from numerous sources and have been described before (2, 43). The bacteria were routinely grown in Luria-Bertani (LB) broth or on LB plates (22). Tetracycline was used at 20 μg/ml for Tn*10* insertion mutants. The bacteria were maintained in 15% glycerol at −70°C; a single colony was picked prior to use.

Transfer of Tn*10* insertions through bacteriophage P22-mediated transduction. A large number of Tn*10* insertions into genes with known functions have been mapped on the genome of *S. enterica* serovar Typhimurium LT2 (21). We transferred Tn*10* insertions from *S. enterica* serovar Typhimurium LT2 to *S. enterica* serovar Pullorum RKS5078 by bacteriophage P22-mediated transduction to locate the same genes through homologous recombination. We made P22 lysates from a selected set of Tn*10* insertion mutants of *S. enterica* serovar Typhimurium LT2 by growing a 3-ml overnight culture of these selected Tn*10* mutants in LB broth and inoculating these cultures with P22 at a multiplicity of infection of 1:100, followed by coincubation for 6 h. After removal of the cell debris by centrifugation, the lysates, 10^{11} PFU/ml, were ready for use in transduction.

For transferring the Tn*10* insertions to *S. enterica* serovar Pullorum RKS5078, we spread 100 μl of an overnight culture of *S. enterica* serovar Pullorum RKS5078 and 20 μl of lysate onto an LB plate containing tetracycline. A colony was picked up and restreaked on another tetracycline plate for single-colony isolation. One colony from the second tetracycline plate was used for phenotype tests and mapping.

Enzymes and chemicals. I-*Ceu*I, *Avr*II, and *Spe*I were purchased from New England Biolabs; *Xba*I and proteinase K were from Boehringer Mannheim. [^{32}P]dCTP was from New England Nuclear. Most other chemicals were from Sigma Chemical Co.

PFGE methods and genomic mapping. Preparation of intact genomic DNA, endonuclease cleavage of DNA in agarose blocks, and separation of the DNA fragments by pulsed-field gel electrophoresis (PFGE) were done as described previously (22, 29). PFGE was performed with the Bio-Rad CHEF Mapper or Bio-Rad CHEF DRII electrophoresis system. For PFGE, we normally use three cycles of conditions: the first for general separation at 30 s of ramping to 90 s for 16 h at full voltage and a buffer temperature of 12°C; the second for zooming in on crowded areas of small bands at 3 s of ramping to 6 s; and the third for zooming in on crowded areas of larger bands at pulsing times based on the sizes of the bands. The total run times for the second and third cycles are usually 6 to 12 h, depending on the extent of the separation. Most runs were carried out at a 120° angle. For very crowded areas of bands, a 150° angle was used.

For determining the sizes of DNA fragments on the PFGE gel, we most often used only a λ ladder (New England Biolabs) as size markers, but in many cases we also used bacterial genomic DNA cleaved with an endonuclease as markers. Among the ones we often used were *S. enterica* serovar Typhimurium LT2 and *S. enterica* serovar Typhi Ty2 DNA cleaved with *Xba*I, *Avr*II, or *Spe*I; the sizes of these fragments had been determined previously (21, 24). These markers significantly improved the precision of the size estimation. Genomic mapping methods with I-*Ceu*I have been described previously (19) and further optimized (25). The technique of double cleavage and end labeling was also described previously (21).

RESULTS

I-*Ceu*I cleavage of *S. enterica* serovar Pullorum RKS5078 genomic DNA. I-*Ceu*I is an intron-encoded endonuclease (10, 31, 32), which cleaves DNA within bacterial *rrl* genes and thus determines the copy number and genomic distributions of *rrl* genes (19). Cleavage of genomic DNA by this endonuclease generated seven fragments in *S. enterica* serovar Pullorum RKS5078 with sizes similar to those of *S. enterica* serovar Typhimurium (21). As our previous work has demonstrated that I-*Ceu*I fragments of similar sizes are homologous among all *Salmonella* species, we designated the seven I-*Ceu*I fragments of *S. enterica* serovar Pullorum with the same letters as in *S. enterica* serovar Typhimurium based on their sizes. The order of the seven I-*Ceu*I fragments in *S. enterica* serovar Typhimurium is ABCDEFG (21). However, the order in *S. enterica* serovar Pullorum RKS5078 was different (Fig. 1A). As shown in Fig. 1A, the partial I-*Ceu*I cleavage products in *S. enterica* serovar Pullorum RKS5078, C+E, D+E, D+F, etc., determined the order FDEC; results from several experiments eventually determined the order as ABFDECG (see the I-*Ceu*I map in Fig. 2).

Cleavages of *S. enterica* serovar Pullorum RKS5078 genomic DNA with *Xba*I, *Avr*II, and *Spe*I. Taking advantage of the *Xba*I and *Avr*II cleavage sites within the Tn*10* DNA sequence, we located the genes in which Tn*10* had inserted by cleavage with *Xba*I and *Avr*II and PFGE separation. Most of the Tn*10* insertions transferred from *S. enterica* serovar Typhimurium were inserted at homologous sites in the genome of *S. enterica* serovar Pullorum RKS5078, as confirmed by phenotype tests, such as auxotropism (18) and loss of motility (17). Most transductants tested had the expected phenotype. A small number of Tn*10* insertions were not inserted into homologous sites; we called them anonymous Tn*10* insertions. These anonymous Tn*10* insertions were also useful, however, for determining the neighboring relationships of some fragments from *Xba*I, *Avr*II, and *Spe*I cleavages. Fig.ures 1B and 1C show *Xba*I and AvrII cleavages, respectively, of *S. enterica* serovar Pullorum RKS5078 and representative Tn*10* insertion mutants.

Unlike *Xba*I or *Avr*II, *Spe*I does not cut the Tn*10* sequence. However, the *Spe*I fragments that have Tn*10* insertions can be recognized by a size increase (9.3 kb). This feature is sometimes very advantageous for reliably assigning a Tn*10* to a certain genomic location by the increased size of an *Spe*I fragment, whereas in the case of *Xba*I and *Avr*II, a Tn*10* inserted within a few kilobases of the end of a fragment is often very

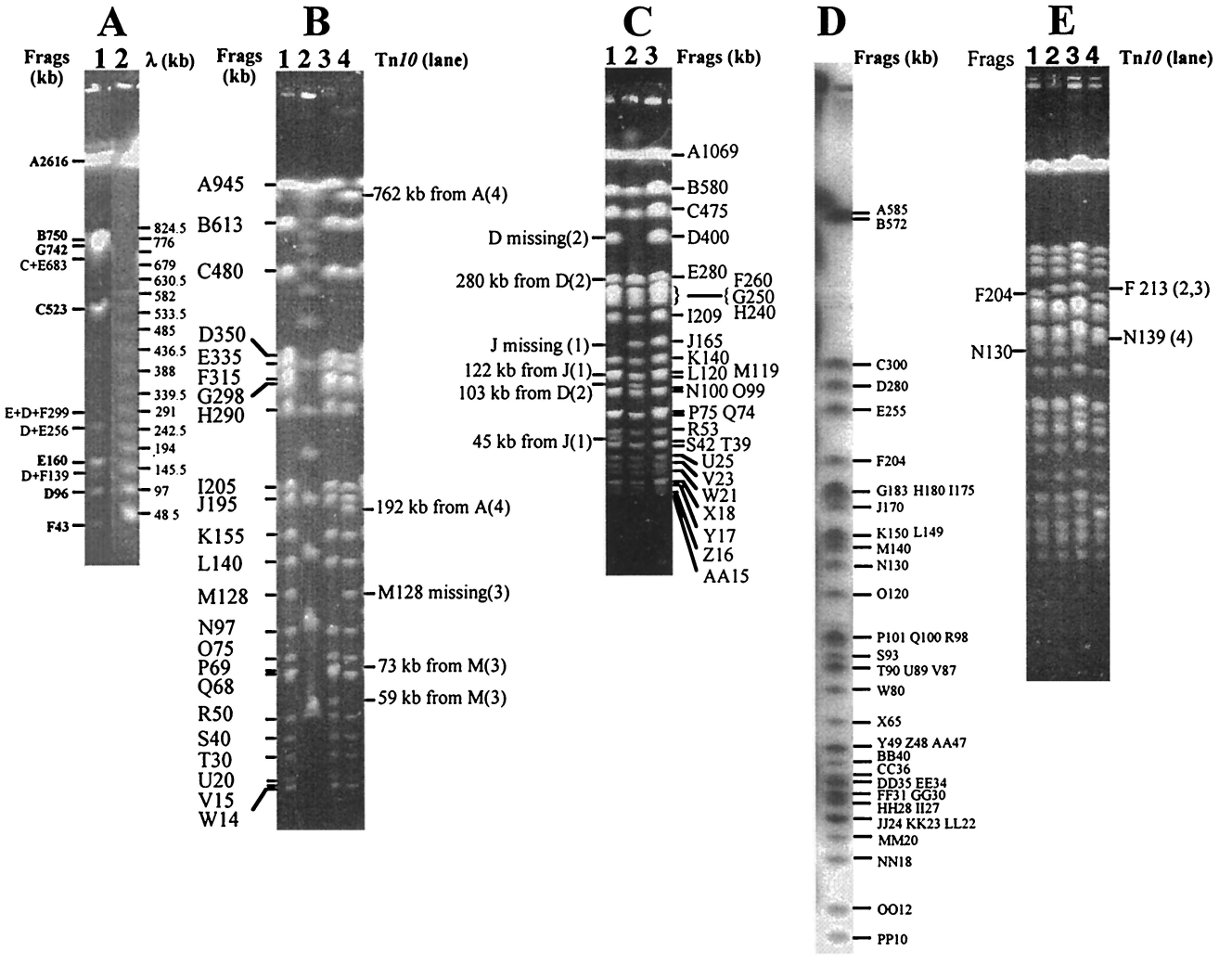


FIG. 1. Endonuclease cleavages of genomic DNA of *S. enterica* serovar Pullorum RKS5078. (A) I-*Ceu*I cleavage pattern. Lanes: 1, *S. enterica* serovar Pullorum RKS5078; 2, λ DNA concatemer as DNA size markers. (B) *Xba*I cleavage patterns of *S. enterica* serovar Pullorum RKS5078 and representative Tn*10* insertion mutants. Lanes: 1, *S. enterica* serovar Pullorum RKS5078; 2, λ DNA concatemer; 3, an anonymous Tn*10* insertion in fragment (Frag) M (128 kb); 4, Tn*10* insertion in *dadB*. (C) *Avr*II cleavage patterns of *S. enterica* serovar Pullorum RKS5078 and representative Tn*10* insertion mutants. Lanes: 1, an anonymous Tn*10* insertion in J (165 kb); 2, Tn*10* insertion in *ompC*; 3, *S. enterica* serovar Pullorum RKS5078. (D) Radioautograph of end-labeled *Spe*I cleavage products of *S. enterica* serovar Pullorum RKS5078. (E) *Spe*I-cleaved genomic DNA of *S. enterica* serovar Pullorum RKS5078 and representative Tn*10* insertion mutants. Lanes: 1, *S. enterica* serovar Pullorum RKS5078; 2, Tn*10* insertion in *nadA*; 3, Tn*10* insertion in *bio*; 4, Tn*10* insertion in *aspC*. See the map locations of these genes in Fig. 2.

difficult to locate on the genome. *Spe*I cleaved genomic DNA of *S. enterica* serovar Pullorum RKS5078 into 42 fragments (Fig. 1D). Fig.ure 1E shows examples of Tn*10* insertion mutants cleaved with *Spe*I.

Genome map of *S. enterica* serovar Pullorum RKS5078. A genome map was then constructed after all cleavage and Tn*10* results were summarized (Fig. 2). This map starts with *thr* as 0 kb and goes clockwise. On the map, we present genomic locations for 72 genes; locations for 30 additional genes are not shown for a cleaner presentation. The location for *oriC* (at 3,975 kb from *thr*) was inferred from the *Escherichia coli* K-12 map by its distances from *uncA* (*atpA*) and *rrlC*. The first four genes, *thr*, *carB*, *leu*, and *pan*, had the same order and spacing among them as in *S. enterica* serovar Typhimurium LT2. The next gene clockwise on the map is *rrlH*/*G*; it is a hybrid between *rrlH* and *rrlG* created by homologous recombination, which resulted in the inversion of I-*Ceu*I A. All genes between *rrlH*/*G* and *rrlG*/*H*, covering 2,616 kb (kb 291 to 2907) were in the reverse order (inversion 1) relative to those in *S. enterica* serovar Typhimurium LT2 except for the genes between *hisA* and *putA* (not inclusive), where there was another inversion of about 1,100 kb (inversion 2). A third inversion (inversion 3) was found between *rrlD* and *rrlE*, resulting in another pair of hybrid *rrl* genes, *rrlE*/*D* and *rrlD*/*E*. Within this third inversion, there was a translocation: I-*Ceu*I D, which is between I-*Ceu*I C and E in *S. enterica* serovar Typhimurium LT2 and most other *Salmonella* genomes, is now between I-*Ceu*I E and F. This translocation results in three hybrid *rrl* genes: when I-*Ceu*I D "left" the original location, the flanking *rrlC* and *rrlA* would join to become *rrlC*/*A*; when it was inserted to the current

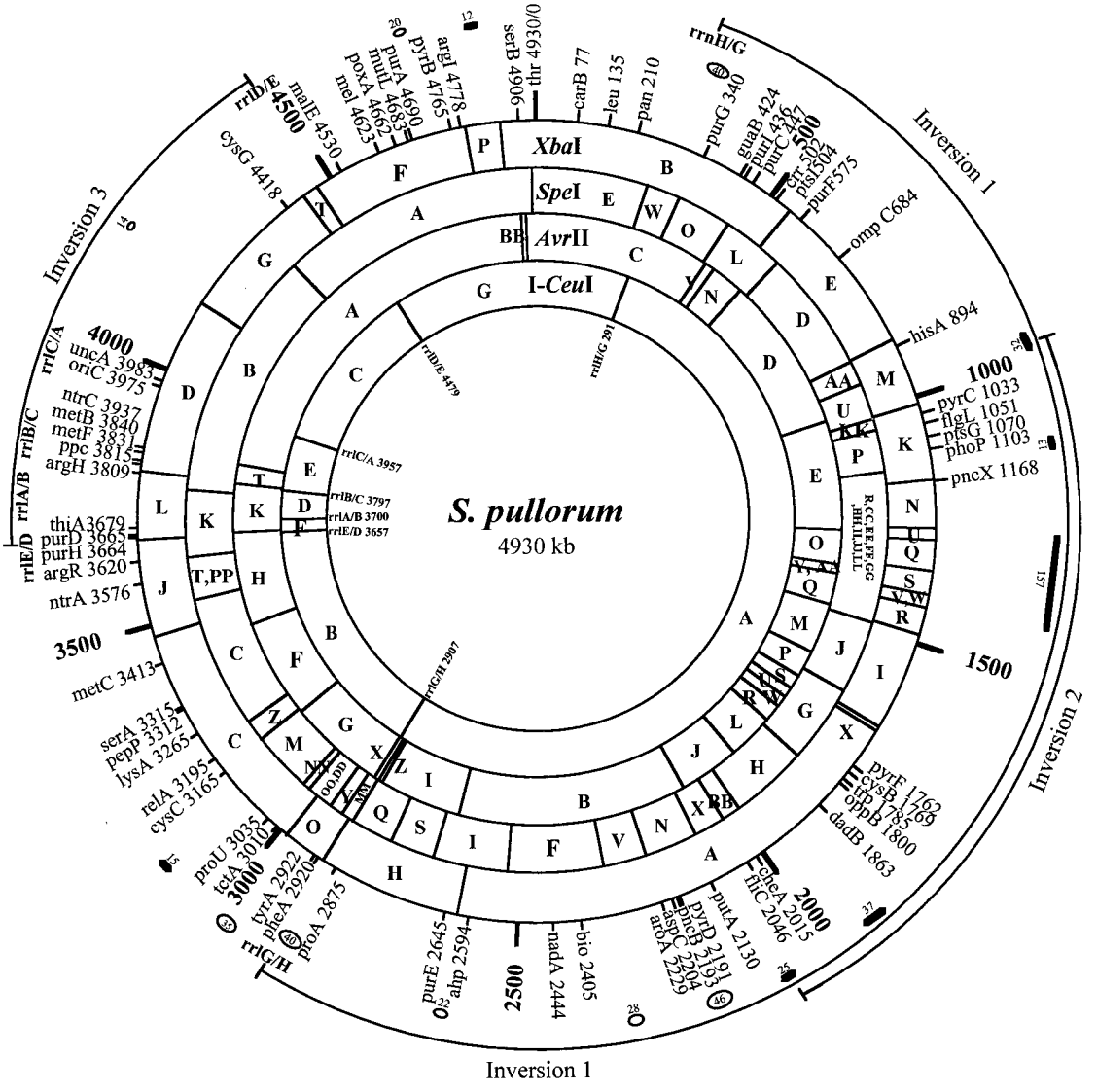


FIG. 2. Genome map of *S. enterica* serovar Pullorum RKS5078. The outermost lines define the ranges of the inversions relative to *S. typhimurium* LT2. Thick short lines show the location and size of insertions (in kilobases). Ovals show the deletions (in kilobases). Some fragments were mapped to the same genomic regions, but their order was not determined.

position, i.e., between I-*Ceu*I E and F, two hybrid *rrl* genes, *rrlB*/*C* and *rrlA*/*B*, would be generated.

Genomic comparisons between *S. enterica* serovar Pullorum and *S. enterica* serovar Typhimurium (21), whose map was greatly refined recently by adding more Tn*10* insertions and the *Spe*I data to improve the resolution (S.-L. Liu, unpublished data), revealed seven areas with increased physical distances between genes, which we assume to be insertions, including 32 kb between *hisA* and *pyrC*, 13 kb between *phoP* and *pncX*, 157 kb between *pncX* and *pyrF*, 37 kb between *dadB* and *cheA*, 25 kb between *fliC* and *putA*, 15 kb between *proU* and *cysC*, and 12 kb between *argI* and *serB*. The sizes are net increases in genomic DNA. At this stage, we do not rule out the possibilities of simultaneous deletions in the vicinity of the "insertion" area (so the actual insertion could be larger), nor do we have evidence that these increased sizes did not result from translocations of genomic segments from other places of the genome. Some or all of the seven areas of increase might contain several smaller insertions. Interestingly, two insertions, one between genes *hisA* and *pyrC* (32 kb) and one between *fliC* and *putA* (25 kb), were found in about the same places as the crossover leading to inversion 2, suggesting the possibility that the two insertions have a high level of sequence similarity and are the actual sites of homologous recombination leading to inversion 2.

Genomic comparisons between *S. enterica* serovar Pullorum and *S. enterica* serovar Typhimurium also revealed eight areas with reduced distances between genes, which we suppose to be genomic deletions, including 40 kb between *rrlH*/*G* and *purG*, 46 kb between *pyrD* and *pncB*, 28 kb between *aroA* and *bio*, 22 kb between *ahp* and *purE*, 40 kb between *proA* and *rrlG*/*H*, 35 kb between *tyrA* and *tctA*, 14 kb between *uncA* and *cysG*, and

20 kb between *purA* and *pyrB*. Again, these sizes are net decreases in genomic DNA and we do not rule out any other genomic events (e.g., insertions) that might have occurred in the vicinity of these areas.

Plastic genome structure of *S. enterica* serovar Pullorum. Having seen the extraordinary genome structure in *S. enterica* serovar Pullorum RKS5078 in addition to the other two *S. enterica* serovar Pullorum strains that we previously mapped with I-*Ceu*I (28), we wondered whether different *S. enterica* serovar Pullorum strains would all have different genome structures, a phenomenon we call plastic genome structure, as in the case of *S. enterica* serovar Typhi (27). We carried out I-*Ceu*I analysis on the strains listed in the Materials and Methods section and found other different genome structures among them, although they all had indistinguishable genome sizes. RKS5078 represented the dominant genome type: 12 strains had the same genome structure as RKS5078, with the seven I-*Ceu*I fragments arranged in the order ABFDECG, including RKS5078, 498, 499, 501, 505, 507, 509, 510, 512, 513, 515, and R278. The remaining four strains each represented a distinct genome type, with strain RKS2242 being ABFDCEG, 490 being ABDECFG, 504 being ABECDFG, and 514 being ABDCGEF (Fig. 3).

The previously mapped *S. enterica* serovar Pullorum strains had other genome structures that were not found here, with RKS2266 being ABDCFEG and RKS2246 being ABEDCFG (28). Most cleavage fragments with *Xba*I, *Avr*II, or *Spe*I were identical in size among all 16 strains, indicating that they were a tight phylogenetic group of bacteria (i.e., they were all "*S. enterica* serovar Pullorum"), though with significant variations in size of a small number of cleavage fragments, presumably a result of different genomic inversions or translocations in different strains.

DISCUSSION

We have constructed a genome map of *S. enterica* serovar Pullorum in strain RKS5078 with an average resolution of about 15 kb. On the map, we determined the size and basic structure of the genome, located over 100 genes, revealed three inversions and one translocation, and resolved seven insertions and eight deletions ranging from 12 to 157 kb, relative to *S. enterica* serovar Typhimurium. In many ways, this genome was similar to those of most *Salmonella* species that we have mapped to date, e.g., the size of the genome and the number of *rrl* genes, two features of phylogenetic significance (30). Especially, the lengths of the seven I-*Ceu*I fragments were typical of salmonellas (19, 28). Even gene order was similar: if the seven I-*Ceu*I fragments were aligned to the genome of *S. enterica* serovar Typhimurium, there would be perfect colinearity (except for the inversion within I-*Ceu*I A) with indistinguishable spacing between genes except for the seven insertions and eight deletions. However, several features did clearly distinguish *S. enterica* serovar Pullorum from other salmonellas, the most striking of which was the extraordinary arrangement of the seven I-*Ceu*I fragments. This special arrangement of the seven I-*Ceu*I fragments seen in RKS5078 represented the dominant genome type of *S. enterica* serovar Pullorum. Why did most *S. enterica* serovar Pullorum strains take this particular genome structure?

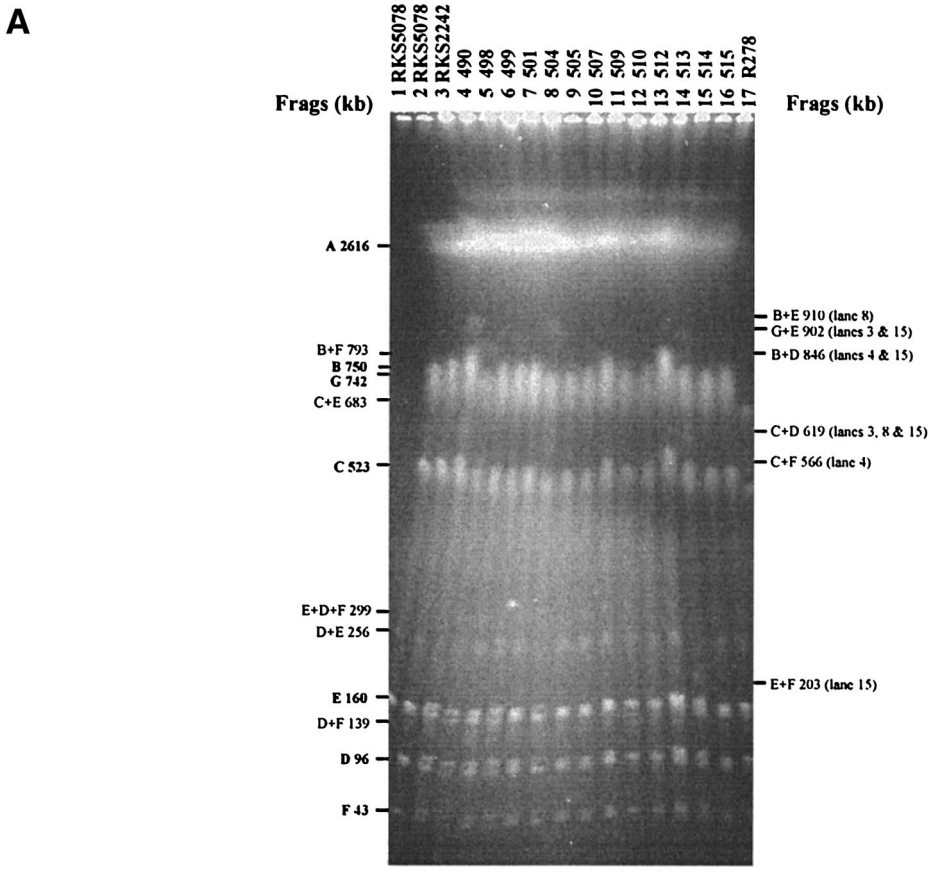
The simplest explanation is rebalancing of the genome by recombination between homologous sites, such as the *rrn* operons, as a compensation mechanism after the genomic balance is disrupted be the 157-kb insertion. This rebalancing, occurring independently in individual *S. enterica* serovar Pullorum cells, creates different genome structures. Cells with better balanced genomes will have larger population sizes. Currently, there are several coexisting genome types of *S. enterica* serovar Pullorum, so we hypothesize that the genome of *S. enterica* serovar Pullorum is still evolving to reach a precise balance through further refinements. The plastic genome stage will last until a cell with a nearly balanced genome appears. This cell will replicate most efficiently, develop lager populations, and eventually replace all other populations. This hypothesized genomic evolution and speciation of *S. enterica* serovar Pullorum is illustrated by a model which we call the adopt-adapt model (Fig. 4).

S. enterica serovar Pullorum has two outstanding biological features, being adapted to fowl and causing only dysentery. Based on the close relatedness and the vast genetic similarity between *S. enterica* serovar Pullorum and other salmonellas such as *S. enterica* serovar Gallinarum, it is reasonable to assume that much of the ability of *S. enterica* serovar Pullorum to cause dysentery might be encoded by the inserted DNA. On the other hand, its host adaptation to fowl might be the result of the genomic deletions: the lost DNA might be present in most other *Salmonella* species that have a broad host range. We are in the process of cloning the seven insertion areas for sequencing and functional analyses. We are also mapping the genome of *S. enterica* serovar Gallinarum, aiming at finding any insertions and deletions. We anticipate finding the set of deletions that are common to *S. enterica* serovar Pullorum and *S. enterica* serovar Gallinarum that may account for both *Salmonella* serovars' becoming adapted to fowl.

The contributions of genomic insertions and deletions to bacterial evolution and speciation are continually being documented (11, 12, 26, 34, 35). Having focused on mainly genomic insertions (18, 23, 24) and inversions and translocations (20, 27), we have for the first time mapped genomic deletions on the genome of *S. enterica* serovar Pullorum. Sequence comparisons of DNA segments that are present in *S. enterica* serovar Typhimurium but absent from *S. enterica* serovar Pullorum with those absent from other host-adapted *Salmonella* species, such as *S. enterica* serovar Typhi, will help in elucidating the mechanisms of host adaptation of salmonellas and in providing new understanding about pathogen-host interaction.

The recent sequence comparisons of pairs of closely related bacteria have provided further insights into and great details about the mechanisms of bacterial divergence, such as *Helicobacter pylori* 26695 and J99 (1, 48), *E. coli* K-12 and O157:H7 (6, 13, 40), *Neisseria meningitidis* z2491 and MC58 (38, 47), and two *Rickettsia* strains (3, 37). Many lines of evidence from these studies have indicated additions of genes as the main cause of divergence. Genomes also lose genes during adaptation to new niches. In addition to deletions, genes decay gradually and lose functions eventually (37). However, genomic balance and rebalancing have not been given due attention as an important factor in genomic evolution and bacterial speciation.

The genome of *S. enterica* serovar Pullorum may have provided a snapshot of salmonellas in the midst of evolution:



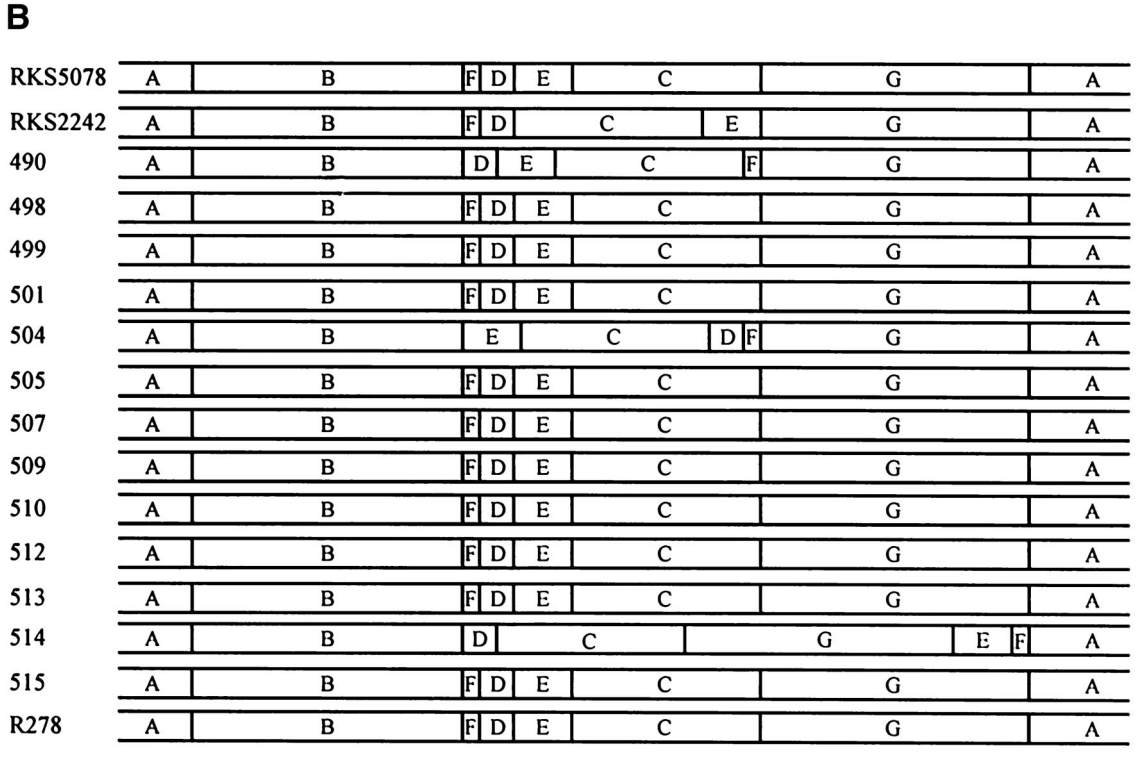


FIG. 3. Population genome structure of *S. enterica* serovar Pullorum. (A) I-*Ceu*I cleavage patterns of wild-type strains of *S. enterica* serovar Pullorum. (B) I-*Ceu*I maps based on data in panel A.

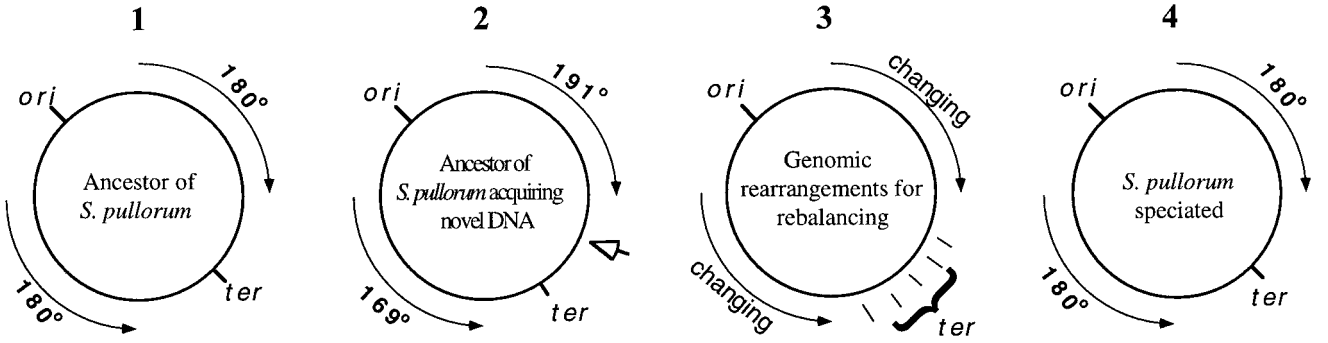


FIG. 4. Hypothesized genomic evolution and speciation of *S. enterica* serovar Pullorum—the adopt-adapt model. Panel 1, Ancestor of *S. enterica* serovar Pullorum having a balanced genome; panel 2, the ancestor having acquiring (adopting) exogenous DNA; panel 3, the genome undergoing adaptive genomic reorganization to reestablish a balance (different genome structures coexist—the plastic genome stage); and panel 4, *S. enterica* serovar Pullorum with a balanced and streamlined genome.

acquiring new genes, losing no-longer-needed ancestral genes, and rearranging the genome for rebalancing. Further analyses of the insertions and deletions of *S. enterica* serovar Pullorum may bring about new discoveries on pathogenesis and host-pathogen interactions of salmonellas, potentially leading to novel strategies of control and utilization of salmonellas and other bacteria. More attention focused on genomic rearrangement, especially the phenomenon of genomic plasticity, may substantially update our understanding of bacterial genomic evolution.

ACKNOWLEDGMENTS

We thank R. K. Selander and C. Poppe for the strains used in this study and Danni Qi, Ning Qi, and Lili Lei for technical assistance.

This study was supported by an operating grant from the Medical Research Council of Canada (MRC, grant GOP-38106) to S.-L.L, an operating grant from the Natural Sciences and Engineering Research Council of Canada (NSERC, grant 216912-00) to S.-L.L, an operating grant from the Canadian Institutes for Health Research (CIHR, grant MOP-47817) to S.-L.L, an NSERC operating grant to K.E.S., NIH grant RO1AI34829 from the National Institute of Allergy and Infectious Diseases to K.E.S., and an MRC operating grant to R.N.J. W.-Q. Liu is a summer student of K.E.S. supported by the Alberta Heritage Foundation for Medical Research.

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