

## Evolutionary Genetic Relationships of Clones of *Salmonella* Serovars That Cause Human Typhoid and Other Enteric Fevers

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Multilocus enzyme electrophoresis was employed to measure chromosomal genotypic diversity and evolutionary relationships among 761 isolates of the serovars *Salmonella typhi*, *S. paratyphi* A, *S. paratyphi* B, *S. paratyphi* C, and *S. sendai*, which are human-adapted agents of enteric fever, and *S. miami* and *S. java*, which are serotypically similar to *S. sendai* and *S. paratyphi* B, respectively, but cause gastroenteritis in both humans and animals. To determine the phylogenetic positions of the clones of these forms within the context of the salmonellae of subspecies I, comparative data for 22 other common serovars were utilized. Except for *S. paratyphi* A and *S. sendai*, the analysis revealed no close phylogenetic relationships among clones of different human-adapted serovars, which implies convergence in host adaptation and virulence factors. Clones of *S. miami* are not allied with those of *S. sendai* or *S. paratyphi* A, being, instead, closely related to strains of *S. panama*. Clones of *S. paratyphi* B and *S. java* belong to a large phylogenetic complex that includes clones of *S. typhimurium*, *S. heidelberg*, *S. saintpaul*, and *S. muenchen*. Most strains of *S. paratyphi* B belong to a globally distributed clone that is highly polymorphic in biotype, bacteriophage type, and several other characters, whereas strains of *S. java* represent seven diverse lineages. The flagellar monophasic forms of *S. java* are genotypically more similar to clones of *S. typhimurium* than to other clones of *S. java* or *S. paratyphi* B. Clones of *S. paratyphi* C are related to those of *S. choleraesuis*. DNA probing with a segment of the *viaB* region specific for the Vi capsular antigen genes indicated that the frequent failure of isolates of *S. paratyphi* C to express Vi antigen is almost entirely attributable to regulatory processes rather than to an absence of the structural determinant genes themselves. Two clones of *S. typhisuis* are related to those of *S. choleraesuis* and *S. paratyphi* C, but a third clone is not. Although the clones of *S. decatur* and *S. choleraesuis* are serologically and biochemically similar, they are genotypically very distinct. Two clones of *S. typhi* were distinguished, one globally distributed and another apparently confined to Africa; both clones are distantly related to those of all other serovars studied.

Of the more than 2,200 serovars (serotypes) of the genus *Salmonella* distinguished in the Kauffmann-White serological scheme of classification on the basis of variation in the somatic lipopolysaccharide (O) and flagellar protein (H) antigens (9, 24-26, 33, 35), only a few are known to be primarily or exclusively limited in host range (host adapted) to humans (see Table 1). Medically, the most important of these is *S. typhi*, the agent of human typhoid fever; others are *S. paratyphi* A, *S. paratyphi* C, and *S. sendai*, all of which cause typhoidlike enteric fevers (20, 54). Additionally, certain strains of *S. paratyphi* B cause human enteric fever, whereas others (designated as *S. java*) produce gastroenteritis in both humans and animals (22, 23). Finally, *S. miami*, which is serologically similar to *S. sendai*, is largely limited to humans but causes gastroenteritis rather than enteric fever (26, 73).

Despite the considerable effort of microbiologists to differentiate and classify *Salmonella* strains by serological,

biochemical, and various other methods, the evolutionary relationships of the human-adapted organisms to one another and to strains of other serovars have remained largely unknown. Consequently, there has been no systematic framework within which to study the evolution of host adaptation and pathogenicity in this group of bacteria. Serotyping is a convenient and epidemiologically useful method of categorizing isolates, but the typological cataloging of surface-expressed antigens can provide little information on the overall genetic relationships of strains of the same or different serovars. It has recently been demonstrated that strains of the same serovar may be distantly related in chromosomal genotype and, conversely, that strains of different serovars may be closely similar in overall genetic character (3, 56). Similarly, bacteriophage types (14, 69), antibiograms (11), and plasmid profiles (17, 19, 41, 43) are useful epidemiological markers and can be medically important for choosing chemotherapeutic regimes, but these properties fail to reflect evolutionary genomic relationships. Attempts to determine relationships among strains and define clones on the basis of variation in biotype characters (2, 48) have had limited success, largely because of the frequent convergence of traits in different phylogenetic lineages (56).

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And while DNA-DNA hybridization has provided a basis for the subspecific classification of the salmonellae (5, 36), it has not contributed to an understanding of genetic relationships among strains within subspecies. Other methods of detecting DNA sequence variation, including the analysis of restriction fragment length polymorphism (RFLP) in plasmid or chromosomal DNA (1, 41, 65, 67, 71), have recently been applied in epidemiological research, but these techniques have as yet had essentially no application in microbial population genetics or systematics.

In recent years, multilocus enzyme electrophoresis has been employed to study the genetic population structure and evolutionary relationships of many types of bacteria (57, 58a), including the salmonellae (3, 52, 56). By assessing electrophoretically demonstrable allelic variations in multiple chromosomal genes encoding metabolic enzymes in large samples of isolates, this technique reveals the genetic structure of natural populations and yields estimates of overall genetic relatedness among isolates, from which phylogenies may be reconstructed (57, 58). In a study of 1,527 isolates of eight common *Salmonella* serovars, Beltran et al. (3) demonstrated a clonal population structure, and for each of six serotypes, from 83% to 96% of isolates were shown to be members of a single clone of worldwide distribution. However, four serovars proved to be polyphyletic, their clones belonging to several evolutionary lineages, some of which are distantly related. Reeves et al. (52) recently concluded that *S. typhi* is a single clone on the basis of enzyme genotypic identity of 26 isolates from diverse geographic regions.

We present here the results of a multilocus enzyme analysis of strains of *S. typhi*, *S. paratyphi* A, *S. sendai*, *S. paratyphi* B, *S. java*, *S. paratyphi* C, and *S. miami*. Comparative genetic data for strains of 22 other serovars, including *S. panama*, *S. typhimurium*, *S. saintpaul*, *S. muenchen*, *S. choleraesuis*, *S. typhisuis*, and *S. decatur*, were utilized to determine the evolutionary positions of the human-adapted strains within the larger context of the salmonellae of subspecies I (33, 36).

## MATERIALS AND METHODS

**Bacterial strains.** Multilocus enzyme genotypes of a total of 1,482 isolates of the 14 serovars listed in Table 1 were analyzed. The strains were obtained from the Institut Pasteur, Paris, France (L. Le Minor); National Veterinary Services Laboratories, Ames, Iowa; the Institut für Veterinärmedizin des Bundesgesundheitsamtes, Berlin, Federal Republic of Germany; the Instituto Nacional de Enfermedades Tropicales in Mexico (D. Bessudo); the National Institute of Public Health, Oslo, Norway (G. Kapperud); the Medical Microbiology Department, University of Dundee Medical School, Dundee, Scotland (R. M. Barker and D. C. Old); the Centers for Disease Control, Atlanta, Ga. (J. J. Farmer III and K. Wachsmuth); the Walter Reed Army Institute of Research, Washington, D.C.; the Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Md.; the Medical College of Virginia, Virginia Commonwealth University, Richmond, Va. (M. Halula); and the National Wildlife Health Research Center, Madison, Wis. (R. M. Duncan).

The isolates had been serotyped at the institutions from which they were obtained or, in the case of some strains supplied by the Centers for Disease Control, at state laboratories. Isolates that differed markedly in multilocus enzyme genotype from the common clones of their assigned

TABLE 1. Antigenic formulas of selected *Salmonella* serovars

Serotypic name	Somatic (O) group	Antigens of indicated type:		
		Somatic (O) <sup>a</sup>	Phase 1 <sup>b</sup>	Phase 2 <sup>b</sup>
<i>S. typhi</i> <sup>c</sup>	O9,12 (D <sub>1</sub> )	9,12,[Vi]	d	
<i>S. paratyphi</i> A	O2 (A)	1,2,12	a	[1,5]
<i>S. sendai</i> <sup>d</sup>	O9,12 (D <sub>1</sub> )	1,9,12	a	1,5
<i>S. miami</i>	O9,12 (D <sub>1</sub> )	1,9,12	a	1,5
<i>S. panama</i>	O9,12 (D <sub>1</sub> )	1,9,12	l,v	1,5
<i>S. paratyphi</i> B	O4 (B)	1,4,[5],12	b	[1,2]
<i>S. java</i> <sup>e</sup>	O4 (B)	1,4,[5],12	b	[1,2]
<i>S. typhimurium</i>	O4 (B)	1,4,[5],12	i	1,2
<i>S. saintpaul</i>	O4 (B)	1,4,[5],12	e,h	1,2
<i>S. muenchen</i>	O6,8 (C <sub>2</sub> )	6,8	d	1,2
<i>S. paratyphi</i> C	O6,7 (C <sub>1</sub> )	6,7,[Vi]	c	[1,5]
<i>S. choleraesuis</i>	O6,7 (C <sub>1</sub> )	6,7	[c]	1,5
<i>S. typhisuis</i> <sup>f</sup>	O6,7 (C <sub>1</sub> )	6,7	[c]	1,5
<i>S. decatur</i> <sup>g</sup>	O6,7 (C <sub>1</sub> )	6,7	c	1,5

<sup>a</sup> Underlining of the O antigen 1 indicates that its expression is connected with phage conversion (see reference 33). Antigenic factors in brackets may be absent.

<sup>b</sup> The genes encoding the phase 1 and phase 2 flagellins, long known as *H1* and *H2*, were recently redesignated as *fliC* and *fliB*, respectively. Antigenic factors in brackets may be absent.

<sup>c</sup> Strains can sometimes be induced to express a phase 1 flagellar antigen j by growth in anti-d serum; and strains normally expressing the j antigen occur in natural populations in Indonesia (10). Some Indonesian isolates also are biphasic, expressing a  $Z_{66}$  flagellar antigen (14) that presumably is encoded by a phase 2 locus (10; see text).

<sup>d</sup> Listed as a serotype by Le Minor and Popoff (35) and as a bioserotype by Ewing (9).

<sup>e</sup> Recently classified as *S. paratyphi* B variety *java* (35) or combined with *S. paratyphi* B (9).

<sup>f</sup> In current classifications, variously treated as a serovar (35) or a bioserotype (9).

<sup>g</sup> Listed as a bioserotype by Ewing (9) but combined with *S. choleraesuis* by Le Minor and Popoff (35).

serovars were reserotyped at the Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, the Enteric Bacteriology Section, Center for Infectious Diseases, Centers for Disease Control, or, in some cases, at both institutions.

The numbers and geographic sources of isolates of the 14 serovars studied are listed below by electrophoretic type (ET). A complete list of isolates and their properties will be provided upon request.

***S. typhi*.** There were 334 isolates of seven ETs. Tp 1—Senegal, 48 (Dakar, 47; unspecified, 1); Cameroon, 20; Zaire, 16; Rwanda, 6; Nigeria, 1; Algeria, 2; Egypt, 3; Syria, 1; Iraq, 1; Turkey, 1; Nepal, 2; India, 1; Pakistan, 2; Sri Lanka, 2; Comoros Island, 1; Indonesia, 26; French Guiana, 3; Colombia, 1; Peru, 15; Chile, 73; Mexico, 30; United States, 5; unspecified, 7; laboratory strains, 8. Tp 1a—Zaire, 1. Tp 1b—Egypt, 1. Tp 1c—Mexico, 2. Tp 1d—United States, 1. Tp 2—Senegal, 52 (Dakar, 50; unspecified, 2); Togo, 1. Tp 2a—Dakar, 1.

***S. paratyphi* A.** There were 135 isolates of six ETs. Pa 1—Algeria, 77; Venezuela, 1; Peru, 4; Chile, 8; United States, 22; unspecified, 2; laboratory strains, 2 (ATCC 9150 and SL1023). Pa 1a—North Carolina, 1. Pa 1b—Oregon, 1. Pa 2—Louisiana, 1. Pa 3—Algeria, 9; Guam, 1; United States, 4; unspecified, 1. Pa 4—Algeria, 1.

***S. sendai*.** There were six isolates of five ETs. Se 1—California, 1. Se 2—New Mexico, 1. Se 3—California, 1; Vietnam, 1. Se 4—Japan, 1. Se 5—New Caledonia, 1.

***S. miami*.** There were 63 isolates of eight ETs. Mi 1—United States, 18. Mi 1a—United States, 2. Mi 2—United States, 2; French Guiana, 7; France, 4. Mi 2a—United

States, 2; France, 2. Mi 3—United States, 1; Panama Canal Zone, 1; Puerto Rico, 4; unspecified, 1. Mi 4—France, 9; Senegal, 4; Guadeloupe Island, 1; unspecified, 1. Mi 5—France, 1; French Guiana, 1; unspecified, 1. Mi 6—France, 1.

*S. panama*. There were 96 isolates of 13 ETs. Pn 1—Europe, 48; Americas, 20; Australia, 2; Thailand, 2. Pn 2—United States, 1. Pn 3—Switzerland, 1. Pn 4—Peru, 1. Pn 5—Poland, 1. Pn 6—Panama, 4; United States, 2; Norway, 1. Pn 7—United States, 2. Pn 8—United States, 2. Pn 9—United States, 2. Pn 10—Brazil, 2. Pn 11—Brazil, 3. Pn 12—United States, 1. Pn 13—Norway, 1.

*S. paratyphi B (including S. java)*. There were 123 isolates from a collection studied by Barker et al. (2) of 14 ETs. Pb 1—Europe, 57; Middle East, 2; India, 1; Africa, 1; South America, 2; unspecified, 11. Pb 1a—Europe, 1. Pb 2—Great Britain, 10; Middle East, 1. Pb 2a—Great Britain, 1. Pb 2b—Great Britain, 1. Pb 3—France, 9; Middle East, 2. Pb 3a—France, 2. Pb 4—France, 4; Great Britain, 3. Pb 5—France, 5; Great Britain, 5. Pb 5a—France, 1. Pb 5b—France, 1. Pb 5c—France, 1. Pb 6—Great Britain, 1. Pb 6a—Africa, 1.

*S. typhimurium*. There were 340 isolates of 17 ETs. Tm 1—Americas, 186; Europe, 53; other regions, 19. Tm 2—United States, 4; Malaysia, 1. Tm 3—Norway, 1; Sweden, 1. Tm 5—Finland, 1. Tm 7—United States, 2. Tm 9—United States, 14; Europe, 3. Tm 10—Europe, 4; Thailand, 5. Tm 11—United States, 5. Tm 12—Norway, 26; France, 1. Tm 13—Panama, 1; Australia, 1; Mongolia, 1. Tm 14—United States, 1. Tm 15—United States, 1. Tm 16—Yugoslavia, 1. Tm 17—United States, 1. Tm 21—Mexico, 1. Tm 22—Europe, 4. Tm 23—United States, 2.

*S. muenchen*. There were 72 isolates of six ETs. Mu 1—Americas, 31; France, 14. Mu 1a—France, 1. Mu 2—United States, 9; France, 10. Mu 3—France, 4. Mu 4—Mexico, 1; United States, 1. Mu 4a—France, 1.

*S. saintpaul*. There were 34 isolates of four ETs. Sp 1—United States, 1; Mexico, 1; France, 3. Sp 2—United States, 1. Sp 3—United States, 1; France, 26. Sp 4—United States, 1.

*S. paratyphi C*. There were 100 isolates of nine ETs. Pc 1—France, 12; Burkina-Faso, 4; Nigeria, 1; Rwanda, 1; Senegal, 5; Egypt, 4; Gabon, 1; Madagascar, 8; Ivory Coast, 1; Korea, 2; Far East, 1; United States, 11; Canada, 1; British Guiana, 1; unspecified, 4; laboratory strains, 3. Pc 1a—France, 3; Madagascar, 1. Pc 2—France, 9; Burkina-Faso, 3; Ivory Coast, 1; East Africa, 1; Senegal, 10; Chad, 1; laboratory strain, 1. Pc 2a—France, 1; Burundi, 1. Pc 3—United States, 1. Pc 4—France, 4. Pc 5—France, 1. Pc 6—Ivory Coast, 1. Pc 7—Ivory Coast, 1.

*S. choleraesuis*. There were 161 isolates of 11 ETs. Cs 1—United States, 68; Canada, 2; Argentina, 1; Brazil, 1; France, 7; Spain, 1; Norway, 1; Belgium, 1; Poland, 5; Romania, 7; Sweden, 1; Switzerland, 1; Yugoslavia, 4; Cameroon, 6; Egypt, 1; Madagascar, 3; New Caledonia, 1; Australia, 3; Tahiti, 4; Indonesia, 1; Thailand, 2. Cs 2—Thailand, 3. Cs 4—United States, 3; Sweden, 1. Cs 6—Switzerland, 1. Cs 7—Senegal, 1. Cs 8—United States, 1. Cs 9—United States, 10; France, 1; Vietnam, 8; Philippines, 1; unspecified, 2. Cs 10—Senegal, 1. Cs 11—Thailand, 3. Cs 12—United States, 2. Cs 13—Australia, 2.

*S. typhisuis*. There were six isolates of three ETs. Ts 1—Continental United States, 3; Hawaii, 1. Ts 2—United States, 1. Ts 3—United States, 1.

*S. decatur*. There were 12 isolates of three ETs. Dt

1—United States, 8; Canada, 1; France, 1. Dt 2—Togo, 1. Dt 3—Togo, 1.

**Electrophoresis of enzymes.** Methods of lysate preparation, protein electrophoresis, and selective enzyme staining have been described by Selander et al. (57). Twenty-four enzymes encoded by chromosomal genes were assayed in all isolates: isocitrate dehydrogenase, aconitase, carbamylate kinase, adenylate kinase, acid phosphatase 1, acid phosphatase 2, 6-phosphogluconate dehydrogenase, phosphoglucose isomerase, nucleoside phosphorylase, catalase, hexokinase, leucylglycyl-glycine peptidase 1, leucylglycyl-glycine peptidase 2, phenylalanyl-leucine peptidase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, mannitol-1-phosphate dehydrogenase, glucose dehydrogenase, phosphoglucomutase, glutamate dehydrogenase, indophenol oxidase, mannose-6-phosphate isomerase, glutamic-oxaloacetic transaminase, and shikimate dehydrogenase.

Except for the addition of shikimate dehydrogenase, this is the same panel of enzymes previously assayed by Beltran et al. (3).

Electromorphs (allozymes) of each enzyme were equated with alleles at the corresponding structural gene locus, and an absence of enzyme activity was attributed to a null allele; all nulls were verified by analysis of freshly prepared lysates. Distinctive combinations of alleles (multilocus genotypes) were designated as ETs (57).

**Statistical analyses.** Genetic diversity at an enzyme locus among ETs was calculated from allele frequencies as  $h = n[1 - \sum x_i^2]/(n - 1)$ , where  $x_i$  is the frequency of the  $i$ th allele,  $n$  is the number of ETs, and  $h$  is the genetic diversity for a single locus (44, 57). Mean genetic diversity ( $H$ ) is the arithmetic average of  $h$  values over all loci.

Genetic distance between pairs of ETs was expressed as the proportion of the enzyme loci assayed at which dissimilar alleles occurred (mismatches). Cluster analysis was performed by the average-linkage method from a matrix of pairwise genetic distances between ETs.

**RFLP in *S. typhi*.** To further define the genetic distinction between the clones Tp 1 and Tp 2 identified by multilocus enzyme electrophoresis, we analyzed variation in the RFLP pattern of the rRNA operons (29, 63) among 10 strains from several geographic regions.

Chromosomal DNA was prepared from cultures by the method of Smith and Selander (60) and 0.5  $\mu$ g was digested with *EcoRI* (with the addition of 4 mM spermidine), electrophoresed on a 0.8% agarose gel, and transferred by capillary action to a nylon membrane (Hybond N; Amersham, Ltd.) by standard methods (39). The rRNA-operon probe was obtained from plasmid pT711 (a gift from L. Lindahl and J. Zengel), which contains the 5.5-kilobase *BclII* fragment of the *rrnB* operon of *Escherichia coli* cloned into the *BamHI* site of plasmid pT7 (Bethesda Research Laboratories, Inc.). The probe was a 3.8-kilobase *PvuII* restriction fragment from pT711 containing the *rrnB* 16S gene, the glutamate tRNA<sup>2</sup> gene, and the promoter-proximal half of the 23S gene. It was isolated by electrophoresis, extracted with GeneClean (Bio 101, Inc.), and nick translated to a specific activity of at least  $10^8$  cpm/ $\mu$ g, all by standard protocols (39).

**DNA probe for Vi capsular antigen genes.** An 8.6-kilobase *EcoRI* fragment of the *viaB* region of the *Citrobacter freundii* (WR7004) chromosome that is specific for genes determining the structure of the polysaccharide Vi antigen (53) was used as a probe to ascertain, by colony blotting, whether the failure of certain strains of *S. typhi* and *S. paratyphi C* to express Vi antigen is a result of gene regulation or an

absence of the *viaB* genes. The methods used in probing are described by Rubin et al. (53).

## RESULTS

**Evolutionary relationships.** A comparison of allele profiles for the 24 enzyme loci assayed in 761 isolates of the seven *Salmonella* serovars *S. typhi*, *S. paratyphi* A, *S. paratyphi* B, *S. java*, *S. paratyphi* C, *S. sendai*, and *S. miami* identified 50 distinctive ETs (Table 2). Comparable data were available for isolates of 22 other serovars, including the eight studied by Beltran et al. (3), *S. typhimurium*, *S. heidelberg*, *S. enteritidis*, *S. newport*, *S. derby*, *S. infantis*, *S. dublin*, and *S. choleraesuis*, and 14 additional ones, *S. panama*, *S. muenchen*, *S. saintpaul*, *S. decatur*, *S. typhisuis*, *S. montevideo*, *S. agona*, *S. gallinarum*, *S. pullorum*, *S. anatum*, *S. rubislaw*, *S. wien*, *S. senftenberg*, and *S. thompson*. Relationships among the ETs of all 29 serovars were examined by average-linkage clustering from a matrix of pairwise genetic distances (data for 15 of the serovars and dendrogram not shown).

Multilocus enzyme genotypes of the ETs of the seven human-adapted serovars and seven other serovars whose isolates showed moderate to high levels of relationship with those of one or more of the human-adapted serovars are presented in Table 2. Among the 1,482 isolates of the 14 serovars, 106 distinctive ETs were distinguished. Estimates of overall genetic distances among these ETs are summarized in the dendrogram presented in two parts in Fig. 1 and 2. On the assumption that rates of evolution of metabolic enzyme genes have been more or less constant in all lineages, the dendrogram may be interpreted as a phylogeny of chromosomal genomes (44, 47).

**Clonal structure of populations.** Following Beltran et al. (3) and Selander et al. (56), we consider that ETs mark clones (see Discussion). Certain clones differing in multilocus enzyme genotype from other, generally more common, clones at only a single locus (especially when the difference involved a null allele) are designated subclones and identified by letter (Table 2).

***S. typhi*. (i) Clonal diversity.** Among 334 isolates of *S. typhi*, we distinguished seven ETs, marking two clones, Tp 1 and Tp 2, and five subclones (Table 2). Tp 1 and Tp 2 differ in alleles at two enzyme gene loci (acid phosphatase 1 and mannitol-1-phosphate dehydrogenase) and are also distinguishable by the RFLP pattern of their rRNA operons (see below).

Tp 1 is the predominant clone of *S. typhi* worldwide, being represented by 275 isolates (82.3% of the total number) from Africa, Eurasia, Southeast Asia, and North and South America. Four minor variant genotypes, each differing from Tp 1 at a single gene locus and represented by only one or two isolates, were designated as subclones (Table 2). The genotype of subclone Tp 1c resembles that of Tp 2 in having a null allele at the acid phosphatase 2 locus, but because the two isolates of Tp 1c were recovered in the course of an epidemic caused by Tp 1 in Mexico in 1972–1973, we interpret them as members of a subclone of Tp 1 rather than of Tp 2.

Tp 2 was represented by 53 isolates (15.8% of the total number), all from Africa; 50 isolates were from Dakar, Senegal, two were from unspecified localities in Senegal, and 1 was from Togo. A subclone, Tp 2a, which differs from Tp 2 in having a null allele at the phenylalanyl-leucine peptidase locus, was represented by a single isolate from Dakar.

**(ii) RFLP pattern of rRNA operons.** When applied to the

*EcoRI*-digested chromosomal DNA of 10 isolates of *S. typhi*, a probe derived from the *rrnB* operon of *E. coli* annealed to a total of 13 restriction fragments (Fig. 3). All five strains of Tp 2 analyzed had the same distinctive RFLP pattern, characterized by the presence of fragments B and E and the absence of fragment C. In contrast, the pattern varied among the five strains of Tp 1 analyzed; all five strains shared eight fragments, but two of the isolates had two fragments (A and D) that were not present in the pattern of the other three strains of Tp 1 or in that of the strains of Tp 2. These two isolates also lacked two other fragments that were shown by the other three isolates of Tp 1.

**(iii) Co-occurrence of Tp 1 and Tp 2.** Isolates of Tp 1 and Tp 2 have been recovered in about equal numbers in Dakar, Senegal, where some individual infections apparently involve both clones. For each of two patients, the culture received from the Institut Pasteur contained organisms of both Tp 1 and Tp 2, which were isolated by single-colony plating.

**(iv) Clonal identities of laboratory strains.** Seven of the eight laboratory strains of *S. typhi* examined, including the widely used Ty2, represented the common clone Tp 1, but Rubin-Kopecko strain 643B (WR4226) differed from all isolates of Tp 1 and Tp 2 in having a 3 (rather than a 5) allele at the acid phosphatase 1 locus and a 7 (instead of a 5) allele at the nucleoside phosphorylase locus. This strain was originally selected for its inability to express Vi antigen after limited conjugal chromosomal exchange with an Hfr strain (WR4018) of *S. typhimurium* (62) and, subsequently, was found to lack the *viaB* region (53). Both the parental Vi-antigen-expressing strain 643 (WR4201) and a Vi-antigen-negative derivative, 643A (WR4205), with a spontaneous mutation at the *viaA* locus (53) are typical examples of Tp 1. The acid phosphatase 1 and nucleoside phosphorylase alleles of strain 643B are those of the common clone of *S. typhimurium*, Tm 1, to which the widely used laboratory strain LT2 and its derivatives belong.

**(v) Strains of *S. typhi* lacking Vi-antigen genes.** In our sample of 334 isolates of *S. typhi*, 6 isolates of Tp 1 and 4 isolates of Tp 2 were phenotypically Vi negative. When probed for the *viaB* region (53), four Tp 1 isolates and three Tp 2 isolates were positive, but DNA from the other two isolates of Tp 1 and from one isolate of Tp 2 failed to hybridize with the probe, thus demonstrating that a small proportion of *S. typhi* strains do not carry the Vi-antigen genes. There was nothing distinctive in the multilocus genotypes of these isolates.

**(vi) Lack of association of multilocus genotype with other characters.** Ten Indonesian isolates expressing a z<sub>66</sub> flagellar antigen rather than the usual d or j antigens (13) were, in multilocus enzyme genotype, typical examples of Tp 1, as were 20 other isolates of *S. typhi* from Indonesia.

All phage-typeable (Vi-antigen-positive) isolates of Tp 2 and all but one of the isolates of Tp 1 from Senegal were type A, which is the commonest phage type of isolates of Tp 1 from Africa.

There was an unusual amount of variation in the level of activity (but not the electrophoretic mobility) of catalase among isolates of Tp 1, but this variation was unrelated to subclone multilocus enzyme genotype, Vi-antigen expression, phage type, or geographic source.

**(vii) Evolutionary relationships.** The ETs of *S. typhi* cluster apart from those of other serovars at a genetic distance of about 0.48 (Fig. 1), which means they are distinctive, on average, at about 12 of the 24 loci assayed. None of the

TABLE 2. Allele profiles for 24 enzyme loci in 106 ETs of 14 *Salmonella* serovars

Serovar and ET	No. of iso-lates	RKS refer-ence isolate	Allele at locus for enzyme <sup>a</sup> :																							
			IDH	ACO	CAK	ADK	AP1	AP2	6PG	PGI	NSP	CAT	HEX	LG1	LG2	PLP	MDH	G6P	M1P	GDH	PGM	GLU	IPO	MPI	GOT	SKD
<i>S. typhi</i>																										
Tp 1	275	3333	3	3	3	3	5	11.3	3	3	5	3	0	3	2	4	3	5	3	3	2	4	3	3	3	2
Tp 1a	1	3390	3	3	3	3	0	11.3	3	3	5	3	0	3	2	4	3	5	3	3	2	4	3	3	3	2
Tp 1b	1	3377	3	3	3	3	5	11.3	3	3	5	3	0	3	2	4	3	5	3	3	1.5	4	3	3	3	2
Tp 1c	2	3972	3	3	3	3	5	0	3	3	5	3	0	3	2	4	3	5	3	3	2	4	3	3	3	2
Tp 1d	1	2068	3	3	3	3	5	11.3	3	2	5	3	0	3	2	4	3	5	3	3	2	4	3	3	3	2
Tp 2	53	3320	3	3	3	3	5	0	3	3	5	3	0	3	2	4	3	5	2	3	2	4	3	3	3	2
Tp 2a	1	3449	3	3	3	3	5	0	3	3	5	3	0	3	2	0	3	5	2	3	2	4	3	3	3	2
<i>S. paratyphi A</i>																										
Pa 1	116	2099	3	3	2	3	5	8	3	3	5	0	3	3	3	3.5	3	3	2	3	3.5	3	3	3	3	5
Pa 1a	1	2909	3	3	2	3	5	8	3	3	5	0	3	3	3	0	3	3	2	3	3.5	3	3	3	3	5
Pa 1b	1	2928	3	3	2	3	0	8	3	3	5	0	3	3	3	3.5	3	3	2	3	3.5	3	3	3	3	5
Pa 2	1	2926	3	3	2	3	5	7.5	3	3	5	0	3	3	3	3.5	3	3	2	3	2.5	3	3	3	3	5
Pa 3	15	2907	3	3	2	3	5	8	3	3	5	0	3	4	3	3.5	3	3	2	3	3.5	3	3	3	3	5
Pa 4	1	3734	3	3	2	3	5	8	3	3	5	0	3	3	3	3.5	3	3	2	3	3.5	5	3	3	3	5
<i>S. sendai</i>																										
Se 1	1	2866	3	3	2	3	0	5	3	3	5	0	3	3	3	3.5	3	3	2	3	3.5	3	3	3	3	5
Se 2	1	2867	3	0	2	3	5	8	3	3	5	0	3	3	3	3.5	3	3	2	3	3.5	3	3	3	3	5
Se 3 <sup>b</sup>	2	4372	3	3	2	3	0	11	3	3	5	0	3	3	3	3.5	3	3	2	3	3.5	3	3	3	3	5
Se 4	1	2869	3	3	2	3	5	11	3	3	5	0	3	3	3	3.5	3	3	2	3	3.5	3	3	3	3	5
Se 5 <sup>b</sup>	1	4373	3	3	3	3	5	14	3	3	6	2.5	3	3	3	4	3	3	2	3	2	3	3	3	3	1
<i>S. miami</i>																										
Mi 1	18	2833	2	3	3	3	5	14	3	3	5	2.5	3	6	3	3.5	3	3	2	3	2	3	3	3	3	1
Mi 1a	2	2835	3	3	3	3	5	14	3	3	5	2.5	3	6	3	3.5	3	3	2	3	2	3	3	3	3	1
Mi 2	13	4382	2	3	3	3	5	14	3	3	5	2.5	3	3	3	3	3	3	2	3	2	3	3	3	3	1
Mi 2a	4	4380	3	3	3	3	5	14	3	3	5	2.5	3	3	3	3	3	3	2	3	2	3	3	3	3	1
Mi 3	7	2855	3	3	3	3	5	14	3	3	5	2.5	3	5	3	4	3	3	2	3	2	3	3	5	3	1
Mi 4	15	4374	3	3	3	3	5	14	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	3	5	3	1
Mi 5	3	4381	3	3	3	3	5	14	3	3	5	2	3	4	3	4	3	3	2	3	2	3	3	5	3	1
Mi 6	1	4398	3	3	3	3	5	14	3	3	6	3	3	4	3	4	3	3	2	3	2	3	3	3	3	1
<i>S. panama</i>																										
Pn 1	75	1793	3	3	3	3	5	14	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	3	3	3	5
Pn 2	1	1776	3	3	3	3	5	14	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	3	3	3	1
Pn 3	1	1842	3	3	3	3	5	0	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	5	3	3	5
Pn 4	1	1848	3	3	3	3	4	14	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	3	3	3	5
Pn 5	1	1817	3	3	3	3	5	14	3	3	5	2.5	3	3	3	3	3	3	3	2	3	3	3	3	3	1
Pn 6	7	1803	3	3	3	3	5	14	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	3	5	3	5
Pn 7	2	1777	2	3	3	3	5	14	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	3	3	3	1
Pn 8	2	1829	2	3	3	3	5	14	3	3	5	2.5	3	4	3	4	3	3	2	3	2	3	3	3	3	5
Pn 9	2	1786	2	3	3	3	5	14	3	3	5	2.5	3	4	3	4	3	3	2	3	3	2	3	5	3	5
Pn 10	2	1872	2	3	3	3	5	14	3	3	5	2.5	3	4	3	2	3	3	2	3	2	3	3	3	3	5
Pn 11	3	1808	2	3	3	3	5	14	3	3	5	2.5	3	3	3	4	3	3	2	3	2	3	3	3	3	1
Pn 12	1	1779	2	3	3	3	5	14	1	3	5	2.5	3	3	3	4	3	2	2	3	2	3	3	3	3	1
Pn 13	1	1880	3	3	3	3	5	14	3	3	5	2.5	3	3	3	4	3	3	2	3	2	3	3	3	3	5
<i>S. paratyphi B (including S. java)</i>																										
Pb 1	74	3222	3	5	3	3	3	14	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 1a	1	3294	3	5	3	3	3	14	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 2	11	3249	2	5	3	3	3	14	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 2a	1	3237	2	3	3	3	3	14	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 2b	1	3267	2	5	3	3	3	14	2	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 3	11	3202	2	5	3	3	3	0	2	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	5
PB 3a	2	3211	3	5	3	3	3	0	2	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 4	11	3201	3	3	3	3	5	14	2	3	3	2	3	3	3	3	3	3	5	3	3	3	3	3	3	5
Pb 5	1	3274	3	3	3	3	3	10	2	3	3	1.5	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 5a	1	3218	3	3	3	3	3	10	2	3	3	1.5	3	3	3	2	3	3	3	3	3	3	3	3	3	5
Pb 5b	1	3219	2	3	3	3	3	10	2	3	3	1.5	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 5c	1	3192	2	3	3	3	3	0	2	3	3	1.5	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Pb 6	1	3277	3	3	3	3	3	10.5	3	3	5	3	3	3	3	3	3	3	3	3	2	3	3	3	3	5
Pb 7	1	3215	3	3	3	3	3	14	3	3	5	2	3	3	3	3	3	3	3	3	2	3	3	3	3	1

Continued on following page

TABLE 2—Continued

Serovar and ET	No. of isolates	RKS reference isolate	Allele at locus for enzyme <sup>a</sup> :																								
			IDH	ACO	CAK	ADK	AP1	AP2	6PG	PGI	NSP	CAT	HEX	LG1	LG2	PLP	MDH	G6P	M1P	GDH	PGM	GLU	IPO	MPI	GOT	SKD	
<i>S. typhimurium</i>																											
Tm 1	258	284	3	3	3	3	3	10	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Tm 2	5	345	2	3	3	3	3	10	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Tm 3	2	821	2	3	3	3	3	7	2	3	7	4	3	3	3	3	3	3	3	3	3	2	3	3	3	3	5
Tm 5	1	811	3	3	3	3	3	7	2	3	7	4	3	3	3	3	3	2.5	3	3	2	3	3	3	3	3	5
Tm 7 <sup>c</sup>	2	203	3	3	3	3	3	10	2	3	7	4	4	3	3	3	3	4	3	3	3	3	3	3	3	3	5
Tm 9	17	154	3	3	3	3	3	5	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Tm 10	9	829	3	3	3	3	3	10	2	3	7	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Tm 11	5	147	3	3	3	3	3	10	2	3	7.5	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Tm 12	27	837	3	3	3	3	3	10	2	3	0	0	4	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Tm 13	3	842	3	3	3	3	3	10	2	3	7	4	3	3	3	3	3	2.5	3	3	3	3	3	3	3	3	5
Tm 14	1	149	3	3	3	3	3	10	2	3	7	4	3	3	3	3	2	3	3	3	3	3	3	3	3	3	5
Tm 15	1	350	3	3	3	3	3	10	2	3	7	4	3	3	3	3	1	3	3	3	3	3	3	3	3	3	5
Tm 16	1	1164	3	3	3	3	3	10	2	3	7	4	3	3	3	3	2.5	3	3	3	3	3	3	3	3	3	5
Tm 17	1	151	3	3	3	3	3	10	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	2	3	3	5
Tm 21	1	93	3	3	3	3	3	10	2	1	7	4	3	3	3	3	3	3	3	3	3	3	3	2	3	3	5
Tm 22	4	839	3	3	3	3	3	10	2	4	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Tm 23 <sup>d</sup>	2	4535	3	3	3	3	3	10	2	3	0	4	3.5	3	3	3	3	3	3	3	2	3	3	3	3	3	5
<i>S. muenchen</i>																											
Mu 1	46	4283	3	5	3	3	3	14	2	3	7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Mu 1a	1	4292	2	5	3	3	3	14	2	3	7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Mu 2	19	4288	3	5	3	3	5	14	2	3	5	4	3	3	3	3	3	3	3	2	2	3	3	3	3	3	1
Mu 3	4	4300	2	3	3	3	5	14	3	3	3	3.5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Mu 4	2	4272	3	3	3	3	3	7	3	3	5	3	3	2	3	3	2	3	3	3	3	3	3	3	3	3	5
Mu 4a	1	4306	3	3	3	3	3	7	3	3	5	3	3	2	3	3	2	3	4	3	3	3	3	3	3	3	5
<i>S. saintpaul</i>																											
Sp 1	5	1688	3	3	3	3	3	7	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Sp 2	1	1689	2	3	3	3	3	8.2	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Sp 3 <sup>e</sup>	27	1690	3	3	3	3	3	8.2	2	3	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
Sp 4	1	1686	2	3	3	3	5	10.5	5.5	3	5	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	1
<i>S. paratyphi C</i>																											
Pc 1	60	4587	3	2	3	3	3	0	3	3	3	3	3	3	3	2	3	3	3	4	3	3	3	3	3	3	6
Pc 1a	4	4588	3	2	3	3	0	0	3	3	3	3	3	3	3	2	3	3	3	4	3	3	3	3	3	3	6
Pc 2	26	4586	3	2	3	3	3	0	3	3	3	3	3	3	3	2	3	3	3	4	3	3	3	3	3	2	6
Pc 2a	2	4589	3	2	3	3	0	0	3	3	3	3	3	3	3	2	3	3	3	4	3	3	3	3	3	2	6
Pc 3	1	2506	3	2	3	3	3	0	3	3	3	3	3	3	3	2	3	3	3	2	3	3	3	3	3	3	6
Pc 4	4	4620	3	3	3	3	5	13	3	3	5	3	3	4	3	4	3	3	4	3	2	3	3	3	3	3	1
Pc 5	1	4594	3	2	3	3	5	0	3	3	3	3	3	3	2	3	3	3	3	4	3	3	3	3	3	2	6
Pc 6	1	4617	3	2	3	3	2.5	0	3	3	3	3	3	3	2	3	3	3	3	4	3	3	3	3	3	3	6
Pc 7	1	4623	2	2	3	3	3	0	3	3	3	3	3	3	2	3	3	3	3	4	3	3	3	3	3	3	6
<i>S. cholerae-suis</i>																											
Cs 1	143	1280	4	3	3	3	3	12	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	3	3	3	3	6
Cs 2	15	1248	3	3	3	3	3	12	3	3	3	3	3	4	3	2	3	2.9	3	3	3	3	3	3	3	3	6
Cs 4	4	1233	3	3	3	3	3	12	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	2	3	3	3	6
Cs 6	1	1239	3	3	3	3	3	8.2	3	3	3	3	5	3	3	3	3	2.9	2	3	2	3	3	3	3	3	1
Cs 7	1	1234	3	2	3	3	3	0	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	3	3	3	3	6
Cs 8	1	1287	4	3	3	3	3	12	3	4	3	3	5	4	3	2	3	2.9	3	3	3	3	3	3	3	3	6
Cs 9 <sup>f</sup>	1	1256	3	3	3	3	3	12	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	3	3	3	3	6
Cs 10 <sup>f</sup>	1	4677	3	2	3	3	3	0	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	3	3	3	2	6
Cs 11 <sup>f</sup>	3	3167	3	2	3	3	3	12	3	3	3	3	5	4	3	2	3	2.9	3	3	3	3	3	0	3	3	6
Cs 12 <sup>f</sup>	2	3177	3	3	3	3	3	12	3	3	3	3	5	4	3	2	3	2.9	3	3	4	3	3	3	3	3	6
Cs 13 <sup>f</sup>	2	4640	3	3	3	3	3	11.5	2	3	5	3	3	3	3	3	3	2.9	3	3	3	3	3	3	3	3	1
<i>S. typhisuis</i>																											
Ts 1	4	3134	5	2	3	3	0	0	3	3	3	3	3	3	3	2	3	3	3	3	3	3	0	2	3	3	6
Ts 2	1	3124	5	2	3	3	0	0	3	3	3	3	3	3	0	2	3	3	3	3	3	3	0	2	3	3	6
Ts 3	1	3133	3	2	3	3	5	8	3	3	5	1.5	3	3	0	3	3	3	2	3	2	3	3	3	5	3	1
<i>S. decatur</i>																											
Dt 1	11	4647	3	2	3	3	5	8	3	3	5	1.5	3	3	0	3	3	3	3	2	3	3	3	5	3	1	
Dt 2	1	4646	2	4	3	3	3	8.2	3	3	3	5	3	3	3	3	3	3	2	3	2	3	3	5	3	1	
Dt 3	1	4645	3	3	3	3	3	8.2	3	3	3	5	3	3	3	3	3	3	2	3	2	3	3	3	3	1	

<sup>a</sup> Enzyme locus abbreviations: IDH, isocitrate dehydrogenase; ACO, aconitase; CAK, carbamylate kinase; ADK, adenylate kinase; AP1, acid phosphatase 1; AP2, acid phosphatase 2; 6PG, 6-phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; NSP, nucleoside phosphorylase; CAT, catalase; HEX, hexokinase; LG1, leucylglycyl-glycine peptidase 1; LG2, leucylglycyl-glycine peptidase 2; PLP, phenylalanyl-leucine peptidase; MDH, malate dehydrogenase; G6P, glucose-6-phosphate dehydrogenase; M1P, mannitol-1-phosphate dehydrogenase; GDH, glucose dehydrogenase; PGM, phosphoglucomutase; GLU, glutamate dehydrogenase; IPO, indophenol oxidase; MPI, mannose-6-phosphate isomerase; GOT, glutamic-oxaloacetic transaminase; SKD, shikimate dehydrogenase.

<sup>b</sup> Received from the Institut Pasteur as *S. sendai* but retyped as *S. miami* at the National Veterinary Services Laboratories.

<sup>c</sup> Tm 7 includes an isolate that was erroneously listed as a distinctive ET, Tm 8, by Beltran et al. (3).

<sup>d</sup> Tm 23 was not included in the analysis by Beltran et al. (3).

<sup>e</sup> Sp 3 includes an isolate that was received as *S. heidelberg* and listed as a distinctive ET, He 9, by Beltran et al. (3). It was retyped as *S. saintpaul* at the National Veterinary Services Laboratories and the Centers for Disease Control.

<sup>f</sup> ETs Cs 9 through Cs 13 were not included in the study by Beltran et al. (3).

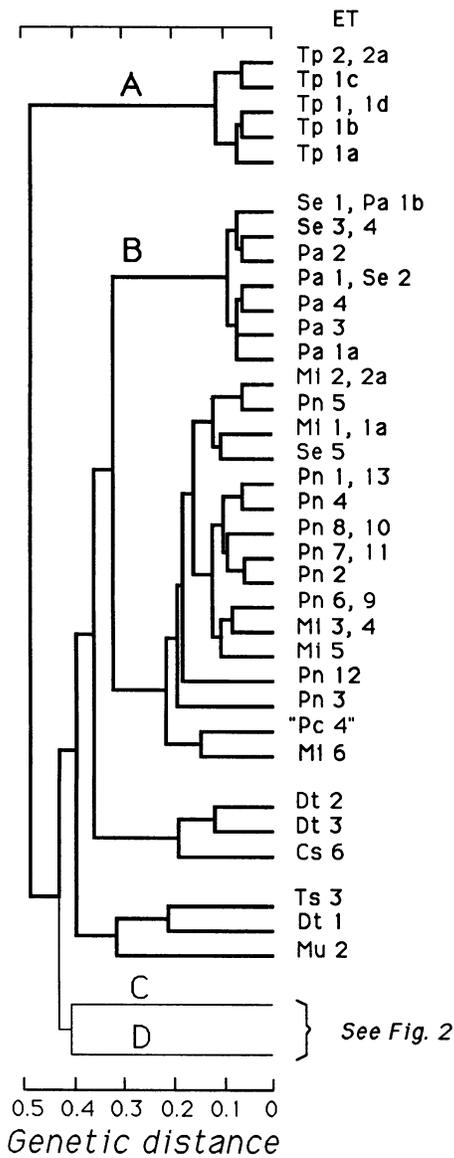


FIG. 1. Part 1 of a dendrogram showing estimated evolutionary genetic relationships among ETs, representing clones, of *Salmonella* serovars causing human typhoid and other enteric fevers and certain other serovars with which they are phylogenetically allied. The dendrogram was generated by the average-linkage method from a matrix of pairwise genetic distances between ETs, on the basis of electrophoretically demonstrable allelic variation at 24 chromosomal enzyme loci. Because the dendrogram was truncated at a genetic distance of 0.04 (corresponding to 1 mismatch in 24), ETs that differ from one another at only a single enzyme locus are not shown as separate branches. The lineage of the cluster of ETs of *S. typhi* (Tp) is labeled A, and that of the cluster containing ETs of *S. paratyphi* A (Pa) and four of the five ETs of *S. sendai* (Se) is labeled B. (Se 5 is a member of the large cluster below cluster B.) Other serovars represented by ETs in this part of the dendrogram are *S. miami* (Mi), *S. panama* (Pn), *S. paratyphi* C (Pc), *S. decatur* (Dt), *S. typhisuis* (Ts), and *S. muenchen* (Mu). Lineages labeled C and D are shown in part 2 of the dendrogram (Fig. 2).

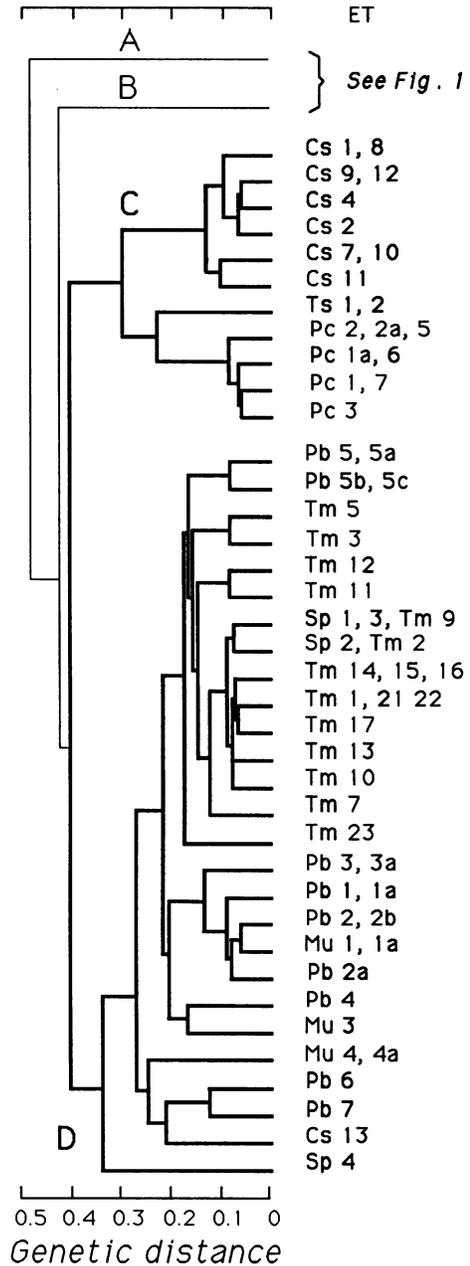


FIG. 2. Part 2 of a dendrogram showing estimated chromosomal genetic relationships among ETs, representing clones, of serovars of *Salmonella* causing human typhoid and other enteric fevers and certain other serovars with which they are phylogenetically allied. The lineage of the cluster containing nine ETs of *S. choleraesuis* (Cs), two ETs of *S. typhisuis* (Ts), and eight ETs of *S. paratyphi* C (Pc) is labeled C; D marks the lineage of the large *S. typhimurium* (Tm), *S. heidelberg* (He) (not shown here; see Beltran et al. [3]), *S. paratyphi* B (including *S. java*) (Pb), *S. saintpaul* (Sp), and *S. muenchen* (Mu), together with one ET of *S. choleraesuis* (Cs 13). Note that one ET of *S. typhisuis* (Ts 3) is a member of a cluster shown in part 1 of the dendrogram (Fig. 1). Lineages labeled A and B are shown in part 1 of the dendrogram (Fig. 1).

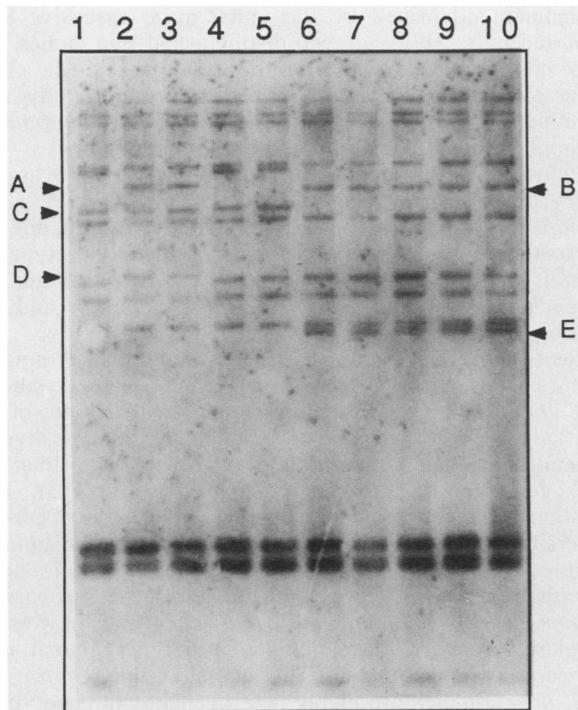


FIG. 3. Southern hybridization of *Eco*RI-digested chromosomal DNA from 10 strains of *S. typhi* representing clones Tp 1 (lanes 1 to 5) and Tp 2 (lanes 6 to 10). The probe contained most of the *rrnB* rRNA operon of *E. coli*. Two patterns are shown by strains of Tp 1. The two strains in lanes 2 and 3 differ from the three strains in lanes 1, 4, and 5 in having fragments A and D and lacking two other fragments (one located above A and one below D). The pattern of Tp 2, which is uniform in the five strains shown here, differs from the patterns of strains of Tp 1 in possessing fragments B and E and in lacking fragment C. Lanes: 1, strain RKS 3333; 2, RKS 3383; 3, RKS 3345; 4, RKS 3421; 5, RKS 3472; 6, RKS 3413; 7, RKS 3468; 8, RKS 3461; 9, RKS 3320; 10, RKS 3417. By analogy with the structure of the *rrnB* operon of *E. coli*, we presume that the two prominent lower fragments are homologous with the internal *Eco*RI fragment and therefore actually represent seven fragments containing tRNA genes.

strains of any other serovar examined is closely related to those of *S. typhi*.

***S. paratyphi* A and *S. sendai*.** (i) **Background.** *S. sendai* resembles *S. paratyphi* A in having O antigens 1 and 12 but differs in expressing O antigen 9 instead of 2. Both have phase 1 flagellar antigen a and phase 2 antigens 1 and 5, but the phase 2 antigens are usually not expressed in *S. paratyphi* A (Table 1). Strains of both serovars reportedly are similar in producing little H<sub>2</sub>S and in failing to ferment tartrate or grow in citrate medium, but *S. sendai* is distinctive in being able to ferment xylose (9).

(ii) **Clonal diversity and relationships.** Six ETs of *S. paratyphi* A, representing four clones and two subclones of the predominant clone Pa 1, were distinguished (Table 2). Two of the clones, Pa 1 and Pa 3, are widespread, perhaps global, in distribution.

Five clones of *S. sendai* were identified. Se 1, Se 2, Se 3, and Se 4 are closely related to one another and to clones of *S. paratyphi* A, but Se 5 is allied with clones of *S. miami* and *S. panama* (Fig. 1). The singular isolate of Se 5 (RKS 4373) was received from the Institut Pasteur as "*S. sendai*" but subsequently was identified (by K.F.) as *S. miami*, although

it was regarded as "atypical" and like *S. sendai* in being citrate negative. The single representative of Se 3 (isolate RKS 4372) was also originally typed as *S. sendai* at the Institut Pasteur and subsequently identified as *S. miami* by K.F.; it is negative for citrate and tartrate and on Stern's medium, all of which traits are characteristic of *S. sendai* (9). We have arbitrarily treated these two isolates as *S. sendai*, but it is clear that identifications of isolates as *S. sendai* or *S. miami* based on biochemical characteristics do not always correspond to the clonal identities indicated by multilocus enzyme electrophoresis.

***S. miami*.** (i) **Background.** Strains designated as *S. miami* have the same antigenic profile as those of *S. sendai* (Table 1) but do not cause enteric fever and are distinctive biochemically, most notably in being able to grow on minimal medium or Simmons citrate agar (30, 31, 35). Additionally, strains of *S. miami* form mannose-sensitive hemagglutinin and type 1 fimbriae, whereas those of *S. sendai* produce a mannose-resistant hemagglutinin (49). Current versions of the Kauffmann-White scheme either follow Kauffmann (24, 25) in listing each form as a separate serovar (35) or classify *S. miami* as a serotype and *S. sendai* as a related bioserotype (9).

(ii) **Clonal diversity and relationships.** There was considerable genotypic diversity among strains of *S. miami*, which represented eight ETs (Table 2). The clones of *S. miami* have no particular evolutionary relationship to those of *S. sendai* or *S. paratyphi* A but, rather, are closely related to strains of *S. panama* (Fig. 1).

The clonal composition of populations varies geographically. All 20 isolates of Mi 1 and Mi 1a were from the United States; all seven isolates of Mi 3 were from North America, Central America, and Puerto Rico; and all 19 isolates of Mi 4, Mi 5, and Mi 6 were from France. In contrast, isolates of Mi 2 and Mi 2a were recovered in both the United States and France.

***S. paratyphi* B and *S. java*.** (i) **Background.** Strains designated as *S. java* have the same serotype as those of *S. paratyphi* B but were distinguished by Kauffmann (22, 23) on the basis of their being *d*-tartrate negative and failing to form a mucoid cell wall (but see Tamura et al. [64]). In current classifications, *S. java* is either combined with *S. paratyphi* B (9) or listed as *S. paratyphi* B variety *java* (35).

(ii) **Clonal diversity and relationships.** Fourteen ETs were distinguished among isolates of *S. paratyphi* B and *S. java* (Table 2). As shown by Selander et al. (56), sensitivity or resistance to colicin M and phage ES18 and the electrophoretic pattern of the rRNA (4), which were the bases for a classification of strains of *S. paratyphi* B and *S. java* recently proposed by Barker et al. (2), individually or in combination fail to mark clones or other meaningful phylogenetic subdivisions. Most *d*-tartrate-negative strains are members of an abundant, globally distributed clone (Pb 1) that is polymorphic for many biotype characters (including *d*-tartrate utilization), phage type, rRNA pattern, and colicin M and phage ES18 sensitivity. This clone is largely responsible for *S. paratyphi* B enteric fever in humans. In contrast, *d*-tartrate-positive strains (*S. java*) occurred in all seven of the clonal lineages identified by population genetic analysis (Fig. 2), but the clonal composition of a large, nonselected sample of isolates from the United States and France indicated that 84% of *d*-tartrate-positive isolates belong to only two clones (Pb 3 and Pb 4) (data not shown). Monophasic strains, all of which are *d*-tartrate positive, represent four closely related clones of a distinctive phylogenetic lineage that clusters with clones of *S. typhimurium* and *S. saintpaul* (Fig. 2). The other

clones of *S. paratyphi* B and *S. java* are somewhat more distantly allied with clones of *S. typhimurium* and are closely related to those of *S. muenchen*.

***S. paratyphi* C. (i) Background.** Strains of *S. paratyphi* C are serologically similar to those of *S. choleraesuis*, *S. typhisuis*, and *S. decatur* but frequently express the Vi antigen, which invariably is lacking in isolates of the other forms (Table 1). Kauffmann (25) reported that the phase 1 flagellar antigenic factor c is serologically somewhat distinctive in each of the four groups, and Le Minor et al. (34) were able to distinguish *S. paratyphi* C, *S. choleraesuis*, *S. choleraesuis* variety *kunzendorf*, and *S. decatur* on the bases of antigenic subfactors and three biochemical tests. *S. decatur*, which differs from the others in giving a positive reaction in Stern's medium (9), has been considered a variety or bioserotype of *S. choleraesuis* (9, 34), but it was recently combined with *S. choleraesuis*, the name "decatour" being withdrawn from the Kauffmann-White scheme (35). *S. typhisuis* is host adapted to swine (66).

**(ii) Clonal diversity and relationships.** Nine ETs, marking seven clones and subclones of *S. paratyphi* C, were distinguished (Table 2). Pc 1, 1a, 2, 2a, 3, 5, 6, and 7 form a tight cluster related to clones of *S. choleraesuis* (Fig. 2). Four monophasic isolates that were identified as *S. paratyphi* C at the Pasteur Institut represent a highly distinctive ET, designated Pc 4 in Table 2 and Fig. 1. As received in the R.K.S. laboratory, two of these isolates (RKS 4620 and RKS 4633) were labeled as Vi positive, but all four isolates failed to hybridize with the *viaB* region probe. When tested by K.F., these four isolates proved to be biochemically unlike either *S. paratyphi* C or *S. decatur* and were classified as untypeable. The Pc 4 clone is unrelated to other clones of *S. paratyphi* C (Fig. 2), being allied, instead, with *S. miami* clone Mi 6 (Fig. 1).

*S. typhisuis* and *S. decatur*, which microbiologists have confused with each other and with *S. choleraesuis*, are each represented in our collection by isolates of three genotypically distinctive clones (Table 2). Both *S. typhisuis* and *S. decatur* apparently are polyphyletic. Clones Ts 1 and Ts 2 of *S. typhisuis* cluster with clones of *S. paratyphi* C and *S. choleraesuis*, but Ts 3 is related to *S. decatur* clone Dt 1 (Fig. 2). Dt 2 and Dt 3 of *S. decatur* are distantly related to Dt 1.

**(iii) Expression of Vi capsular antigen in strains of *S. paratyphi* C.** There was no association of Vi-antigen expression with variation in multilocus genotype among strains of *S. paratyphi* C. We examined 19 Vi-positive and 23 Vi-negative isolates of Pc 1 and 12 Vi-positive and 13 Vi-negative isolates of Pc 2. A total of 70 isolates, representing several ETs, were challenged with the *viaB*-specific probe. Half these isolates had been typed as phenotypically Vi negative, but all except one (RKS 4630) hybridized with the probe.

## DISCUSSION

**Clonal population structure.** In an analysis of strains of eight common serovars, Beltran et al. (3) deduced that the genetic structure of populations of the salmonellae is clonal on the basis of the intercontinental, if not global, distribution of certain multilocus enzyme genotypes and the presence of linkage disequilibrium (nonrandom associations of alleles) between pairs of enzyme loci. Three of the serovars studied, *S. typhimurium*, *S. heidelberg*, and *S. choleraesuis*, are each monophyletic and have one predominant, widely distributed clone. Reeves et al. (52) added *S. typhi* to this list, an

assignment confirmed by the much more extensive data reported here. However, we distinguished two clones and five subclones of *S. typhi* rather than the single clone detected by Reeves et al. (52). The present study also identified *S. paratyphi* C, *S. panama*, and *S. saintpaul* as monophyletic serovars with one predominant clone.

Beltran et al. (3) identified four polyphyletic serovars, *S. derby*, *S. newport*, *S. enteritidis*, and *S. infantis*. *S. paratyphi* B was added to this list by Selander et al. (56), and the present study demonstrated that each of the five serotypes *S. miami*, *S. typhisuis*, *S. decatur*, *S. saintpaul*, and *S. muenchen* is strongly heterogeneous in genotype and, on that basis, apparently polyphyletic.

**Genetic diversity in relation to host adaptation.** A notable feature of variation emerging from population genetic studies of *Salmonella* species is a strong tendency for clones of the host-adapted serovars to be fewer in number and less diverse in multilocus genotype than those of serovars that are pathogenic for a variety of host species (Table 3). This relationship is apparent, for example, when levels of genetic diversity among ETs (representing clones) of the human-adapted serovars *S. typhi*, *S. paratyphi* A, *S. sendai*, and *S. paratyphi* C are compared with those for the serovars *S. typhimurium*, *S. panama*, and *S. muenchen*, each of which is regularly recovered from a wide variety of animal host species as well as humans. That clones of the *S. paratyphi* B-*S. java* complex are highly heterogeneous in genotype is

TABLE 3. Mean genetic diversity per locus (*H*) among clones (ETs) of *Salmonella* serovars in relation to host range

Serovar	Isolates (no.)	Clones (no.)	<i>H</i>
<b>Host adapted</b>			
<i>S. typhi</i>	334	2	0.083
<i>S. paratyphi</i> A	135	4	0.042
<i>S. sendai</i>	6	5	0.162
	5	4 <sup>a</sup>	0.083
<i>S. paratyphi</i> C	100	7	0.150
	96	6 <sup>b</sup>	0.074
<i>S. choleraesuis</i>	161	11	0.120
	159	9 <sup>c</sup>	0.089
<i>S. dublin</i> <sup>d</sup>	206	2	0.042
<i>S. gallinarum</i> <sup>e</sup>	50	4	0.055
Mean 1 <sup>f</sup>			0.0934
Mean 2 <sup>g</sup>			0.0669
<b>Non-host adapted</b>			
<i>S. muenchen</i>	73	4	0.326
<i>S. panama</i>	96	13	0.126
<i>S. saintpaul</i>	34	4	0.188
<i>S. typhimurium</i>	340	17	0.119
<i>S. heidelberg</i> <sup>h</sup>	204	8	0.092
<i>S. derby</i> <sup>h</sup>	349	6	0.258
<i>S. newport</i> <sup>h</sup>	105	13	0.149
<i>S. enteritidis</i> <sup>h</sup>	257	14	0.176
<i>S. infantis</i> <sup>h</sup>	113	4	0.152
Mean			0.1762

<sup>a</sup> Se 5 excluded.

<sup>b</sup> Pc 4 excluded.

<sup>c</sup> Cs 6 and Cs 13 excluded.

<sup>d</sup> Selander et al., unpublished data.

<sup>e</sup> Li and Selander, unpublished data.

<sup>f</sup> Based on higher values for *S. sendai*, *S. paratyphi* C, and *S. choleraesuis*.

<sup>g</sup> Based on lower values for *S. sendai*, *S. paratyphi* C, and *S. choleraesuis*.

<sup>h</sup> From Beltran et al. (3). Estimates of *H* were based on 23 enzyme loci. Other values of *H* in the table are based on 24 loci (see Materials and Methods).

not an exception to this rule, because only a few clones are host adapted and the degree of adaptation may be relatively weak. Diversity among the small number of clones of *S. paratyphi* B that cause enteric fever in humans is very limited indeed, with most of this disease being produced by a single clone, Pb 1 (56). And the same is true for the gastroenteritis-causing *S. miami*, some (but not all) clones of which apparently are weakly adapted to humans (see beyond). If we exclude from consideration the outlying and apparently rare Cs 6 and Cs 13 ETs of *S. choleraesuis*, the clones of this swine-adapted serovar show only a moderate amount of diversity. Other examples are the cattle-adapted serovar *S. dublin*, with only two clones, which are genotypically similar to one another (Selander et al., unpublished data), and *S. gallinarum*, which consists of four closely related clones adapted to chickens (J. Li and R. K. Selander, unpublished data).

Population genetics theory provides two very different explanations for the observed relationship between host range and genotypic diversity, one relating to effective population size and the other to ecological niche breadth. Consider first the effective population size model. Under the assumptions of the neutral mutation theory of molecular evolution (28), the amount of allelic variation at a locus in a finite population at equilibrium between the generation of selectively neutral mutations and their loss through random genetic drift is a direct function of the effective size of the population ( $N_e$ ). For bacteria with a clonal population structure, effective population size may more closely correspond to the total number of extant colonies than to the actual size of the total standing crop of cells (40). At equilibrium, which theoretically may be attained only after a period of time equal to  $2N_e$  generations, smaller populations will maintain lesser degrees of genetic variation than will larger populations. For populations not at equilibrium, the evolutionary effective size is roughly the harmonic mean of the effective size of the population over all generations since its origin. Consequently, if new populations arise from one or a small number of cell lineages, younger populations are expected to be genetically less variable than older populations.

*Salmonella* serovars with strains capable of infecting a variety of different host species may, other things being equal, be expected to maintain larger effective population sizes and, consequently, to carry more genetic variation than serovars that are limited in distribution to humans or single species of animals. It is also probable that many or all of the host-adapted serovars have arisen more recently than the common, broad-host-range serovars. However, because we do not know whether populations are at equilibrium, the two forms of the neutral mutation hypothesis cannot be separately tested.

The ecological niche breadth model of population variation is based on the premise that much or all of the allelic diversity at enzyme and other structural gene loci is adaptive and is maintained by one or more types of balancing selection (28, 44, 46). According to this model, the relatively narrow range of ecological conditions encountered by strains of a host-adapted serovar selects for a corresponding limited amount of genetic diversity. Serovars of this type are expected to be represented by only a small number of closely related, highly specialized clones. But in the case of the ubiquitous serovars, clones of many different genotypes may find ecological niches to which they are especially adapted or there may be selection for general purpose genotypes that are moderately well adapted to a wide range of ecological conditions provided by a variety of host species.

Because of the formidable nature of the problem of estimating either evolutionary effective population size or niche breadth, it will be extremely difficult to test the two hypotheses. However, it should be noted that the apparent selective neutrality or near neutrality of electromorph alleles at bacterial enzyme loci, as demonstrated experimentally and statistically for *E. coli* (15, 16, 72), is compatible with the interpretation that evolutionary effective population size is the major determinant of the amount of protein polymorphism carried by populations. It is also relevant to note that repeated efforts to demonstrate ecological correlates of the amount of genetic variation in populations of higher organisms have yielded little success (45, 55).

**Limited genotypic diversity in *S. typhi*.** Of the 29 common *Salmonella* serovars we have studied by multilocus enzyme electrophoresis (3, 56; this report; Beltran and Selander, unpublished data), *S. typhi* is genotypically among the least heterogeneous (Table 3). Additional evidence of a relatively low level of genetic variation among isolates of *S. typhi* is provided by studies of restriction enzyme digestion patterns of chromosomal DNA from isolates from Chile, Peru, and the United States (38) and by studies of plasmids conferring resistance to chloramphenicol in isolates from Mexico, Vietnam, Thailand, and India (65). It is also reported that *S. typhi* is unusually homogeneous in biochemical characteristics (50).

Most populations of *S. typhi* are monomorphic for the d allele at the *HI* locus encoding the phase 1 flagellin protein. But Indonesian populations are polymorphic for the d allele and a variant j allele, which arises when homologous recombination causes a deletion in the central antigenically determinant part of the d allele (10). Some Indonesian strains also express a  $z_{66}$  flagellar antigen (13), which presumably is encoded by a phase 2 locus (*H2*) (10). Strains with the  $z_{66}$  antigen are known only from Indonesia; extensive surveys have failed to detect the presence of this antigen in populations of *S. typhi* in Madagascar, Africa, the Antilles, and Central and South America (68, 70).

In view of the allelic polymorphism at the *HI* locus and occasional expression of an apparent *H2* gene in the Indonesian population of *S. typhi*, it is noteworthy that all 26 Indonesian isolates we examined, including six strains expressing the  $z_{66}$  factor, were indistinguishable in multilocus enzyme genotype from other members of the globally distributed Tp 1 clone.

The most variable features of *S. typhi* are the response to Vi phages (14) and the RFLP pattern of the rRNA operons. A total of 106 lysotypes has been recognized (7); some lysotypes are cosmopolitan (e.g., types A, B3, and C1), whereas others are regionally confined or are much more common in some geographic areas than in others (e.g., types G and M). Consequently, there is much geographic variation in the frequencies of various lysotypes and groups of lysotypes (14).

As first reported by Altwegg et al. (1) and also demonstrated in the present study, there is considerable RFLP in the rRNA operons of *S. typhi*. The manner in which this variation is apportioned geographically among populations of the globally distributed Tp 1 clone remains to be studied.

**Simultaneous infection by two clones of *S. typhi*.** We obtained evidence that individual humans in Senegal may be infected simultaneously by strains of clones Tp 1 and Tp 2, both of which are endemic and epidemic in that country. Infection with multiple strains of *S. typhi* reportedly is also not uncommon in Lima, Peru, as evidenced by the observa-

tion that strains isolated simultaneously from single patients may differ in plasmid profile (12).

**Vi capsular antigen in *S. typhi* and *S. paratyphi* C.** DNA probing for the Vi antigen structural determinant genes in 10 phenotypically Vi-negative isolates of *S. typhi* indicated that the occasional absence of the antigen may reflect either the absence (three cases) or lack of expression (seven cases) of the genes.

In the case of *S. paratyphi* C, we have demonstrated that the frequent failure of strains in laboratory culture to produce the Vi antigen is, with rare exception (1 of 35 phenotypically Vi-negative isolates probed), not attributable to an absence of the *viaB* gene region (61). Daniels et al. (6) found that strains of *S. paratyphi* C rapidly lose Vi expression when passed on laboratory medium. In view of this finding and the observation that 67 of 78 isolates recovered in the course of an epidemic of enteric fever in South Africa and stored freeze-dried were Vi positive, Daniels et al. (6) suggested that most strains of *S. paratyphi* C have Vi in the host.

Because the Vi antigen occurs in three distantly related phylogenetic lineages of *Salmonella* (*S. typhi*, *S. paratyphi* C, and *S. dublin*) and in *C. freundii*, the inference is that several horizontal gene transfer events involving the *viaB* region have occurred in the evolutionary history of these bacteria. The presence of the *viaB* region in strains of all clones of *S. typhi* and *S. paratyphi* C suggests that it has been in these lineages for long periods. If so, the instability of expression of the Vi antigen in strains of the latter serovar cannot be attributed to a recent acquisition of the genes. In the case of *S. dublin*, however, acquisition of the *viaB* region may have occurred relatively recently, for these genes are confined to a single minority subclone of the predominant, widely distributed clone (Du 1), which, moreover, has a limited distribution, occurring only in Europe and Israel (Selander, unpublished data). All isolates of this subclone have the *viaB* genes, and expression of the Vi antigen is stable. Vi-positive strains of *C. freundii* apparently are rare, and expression of the antigen is highly variable as a result of the presence of an invertible insertion sequence in the *viaB* region (51, 62).

**Evolution of host adaptation and pathogenicity.** From the information on phylogenetic relationships provided by our analysis, tentative inferences may be drawn regarding the evolution of clones that are largely or entirely adapted to humans and the development of the propensity to cause human enteric fever.

*S. paratyphi* A and *S. sendai* evolved from a common ancestral lineage that also gave rise to the clones of *S. panama*, which are agents of gastroenteritis in a broad range of hosts, including humans, and to *S. miami*. With regard to surface serological characters, the simplest hypothesis is that the phase 1 flagellar antigen a is the ancestral condition and was retained in *S. paratyphi* A, *S. sendai*, and *S. miami*, being replaced by the 1,v antigens in *S. panama* (see Table 1). Additionally, the O antigen 9 was lost and the phase 2 flagellin gene (*H2*) was silenced in *S. paratyphi* A. The derivation of *S. paratyphi* A and *S. sendai* from an *S. panama*-like ancestor would have involved both a restriction in host range to humans and the development of invasive ability. These changes presumably occurred in an ancestral clone that very recently differentiated, perhaps geographically, into the clones of *S. paratyphi* A and *S. sendai*.

The serovar *S. miami*, which is predominantly North American in distribution (27), is genotypically heterogeneous and may have arisen several times from various clones

of *S. panama*-like organisms. The predominant clone is Mi 1; isolates of this clone and Mi 2 and 2a were recovered from humans, whereas isolates of clones Mi 3, Mi 4, and Mi 5, which are on a different evolutionary branch (Fig. 1), were obtained from a frog, a fish, and guano. Mi 6, which is on still another branch, was cultured from a blackbird. *S. miami* has also been reported from other types of warm- and cold-blooded animals (8, 18). These data, although limited, suggest that only the first group of clones is adapted to humans. The evolution of *S. miami* (or at least clones Mi 1 and Mi 2) involved adaptation to humans, without the acquisition of characters facilitating invasiveness. The alternative possibility that restriction to humans is the ancestral condition, with *S. panama* and some clones of *S. miami* secondarily evolving broad host ranges, seems less likely. (Note that Se 5 is actually a clone of *S. miami*.)

*S. paratyphi* B (including the *d*-tartrate-positive strains long designated as *S. java*) is a genotypically heterogeneous complex of clones that is related to clones of *S. typhimurium*, *S. heidelberg*, and *S. saintpaul*; serologically, these four serovars differ only in phase 1 flagellar antigens. *S. muenchen* is also part of this complex (59), although it is distinctive in both somatic and phase 1 antigens. Our analysis suggests that *S. paratyphi* B is polyphyletic, with the monophasic clones being very close to *S. typhimurium*, *S. heidelberg*, and the main group of *S. saintpaul* clones. Human adaptation and the ability to cause enteric fever evolved in clone Pb 1, presumably rather recently, since this clone is only weakly differentiated from other clones that cause gastroenteritis and have broad host ranges. No change occurred in the other lineages of *S. paratyphi* B, which, together with *S. typhimurium*, *S. heidelberg*, *S. saintpaul*, and *S. muenchen*, have retained the ecological and pathogenic characteristics of the ancestral condition of the complex.

The clones of *S. paratyphi* C and those of *S. choleraesuis*, together with certain clones of *S. typhisuis* (Ts 1 and Ts 2), apparently shared a common ancestor from which they evolved without modification of the serotype, except for the acquisition of the Vi antigen by *S. paratyphi* C. We suggest that the common ancestor was invasive and already adapted to swine, as are the extant clones of *S. choleraesuis* and certain clones of *S. typhisuis*, which cause swine paratyphoid fever (66). If so, the evolutionary derivation of *S. paratyphi* C would have involved only a shift in host to humans. The occasional occurrence of *S. paratyphi* C in animals suggests that physiological specialization for humans is not as complete as in the case of *S. typhi* or *S. paratyphi* A. It is noteworthy that in some parts of the world humans are a significant secondary host for *S. choleraesuis* (21, 32), which is invasive, producing severe enteric fever with an unusually high mortality rate (73).

*S. typhisuis* apparently is polyphyletic; Ts 3 is genotypically very different from Ts 1 and Ts 2, notwithstanding the serotypic identity and physiological similarity of all three clones.

*S. decatur* is also genotypically heterogeneous. Dt 1 is related to Ts 3, and Dt 2 and Dt 3 are related to Cs 6.

Because no close relative of *S. typhi* was identified by our analysis or that of Reeves et al. (52), there is no basis for speculation regarding the host range and pathogenicity of the ancestral population from which clones of this serovar evolved. The marked distinction in chromosomal genotypes of the clones of *S. typhi* from those of the clones of other serovars indicates that their phylogenetic lineage is old, but both their distinctive characters and close adaptation to

humans could be fairly recent developments. Indeed, the extant clones of *S. typhi* may have arisen so recently that there has not been sufficient time for the mutational or recombinational generation of any large amount of genotypic diversity in enzyme genes or other genes that have moderate to slow evolutionary rates. However, there is the alternative possibility that the relatively low level of genotypic heterogeneity among strains of *S. typhi* is a consequence of a recent episode of periodic selection (37) affecting populations on a global scale.

Frankel et al. (10) have outlined an evolutionary scenario in which *S. typhi* evolved as a specialized human pathogen in Indonesia. According to their hypothesis, it initially was biphasic (d:z<sub>66</sub>), mutations subsequently produced the monophasic condition (d:-) and the j allele at the *H1* locus, and a single subclone of the monophasic d type spread worldwide from Indonesia relatively recently. This hypothesis is appealing primarily because it accounts for the apparent restriction of the biphasic condition to Indonesia. Alternatively, however, the occurrence in Africa of two clones (Tp 1 and Tp 2) marked by a two-locus difference in multilocus enzyme genotype could be advanced as evidence for an African origin of *S. typhi*, with the biphasic condition having been secondarily acquired in one or more cell lineages of the Indonesian population by horizontal transfer of the *H2* gene.

**Classification of the salmonellae.** The Kauffmann-White serological scheme for *Salmonella* strains ostensibly is a diagnostic catalog of antigenic variation rather than a taxonomic classification (24, 25), but implicit in most of its applications is the notion that isolates of a given serovar share many homologous genetic properties in addition to cell surface antigens and are, therefore, in some meaningful sense biological entities. This concept has been strongly fostered by the use of scientific species nomenclature for the serovars. Kauffmann (24) considered each serovar a species, which he defined as "a group of related serofermentative phage-types," but, in fact, the scheme has no adequate provision for determining relatedness among strains in an evolutionary genetic sense.

Like all systems of classification that are both typological and based on a small number of characters, the Kauffmann-White scheme was bound to prove unsatisfactory when methods of detecting multilocus genotypic variation became available and evolutionary population genetic concepts were applied to the study of bacterial phylogeny and classification (42). The reason it has been useful through the years is that the majority of isolates of many of the common serovars (e.g., *S. typhimurium*) are members of a single clone that predominates in natural populations worldwide (3). In these cases, serotypic identity of isolates is, in fact, likely to reflect overall genotypic identity or close similarity. But the numerous cases in which isolates of the same serotype have markedly different chromosomal genotypes and evolutionary relationships (3, 56; this report) invalidate the Kauffmann-White scheme as a general system of biological classification of the salmonellae. As a framework for analyzing variation in plasmids, biochemical characteristics, pathogenicity, and other properties of strains and for studying global epidemiology and the genetic structure and evolution of populations, it is grossly inadequate.

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