Enzyme Polymorphism and Genetic Population Structure in *Escherichia coli* and *Shigella*

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Electrophoretically demonstrable variation in 12 enzymes was studied in more than 1600 isolates of *Escherichia coli* from human and animal sources and in 123 strains of the four species of *Shigella*. All 12 enzymes were polymorphic; and the number of allozymes (mobility variants), which were equated with alleles, averaged 9.3 per locus in *E. coli*. For *Shigella* species, the mean number of alleles was 2.9 per locus. Some 77% of the allozymes recorded in *Shigella* were shared with *E. coli*. A total of 302 unique genotypic combinations of alleles over the 12 loci (electrophoretic types, ETs) was distinguished, of which 279 represented *E. coli* and 23 were *Shigella*. Among electrophoretic types, mean allelic diversity per locus was 0.52 for *E. coli* and 0.29 for *Shigella*. It was estimated that there are, on the average, about 0.3 detectable codon differences per locus between pairs of strains of *E. coli* and *Shigella*, which is roughly equivalent to 1.2 amino acid differences per enzyme. Evidence that the enzyme loci studied are a random sample of the genome is provided by a significant positive correlation between estimates of genetic divergence between pairs of strains obtained by DNA reassociation tests and estimates of genetic distance between the same strains based on electrophoresis.

A principal components analysis of allozyme profiles revealed that the 302 ETs fall into three overlapping clusters, reflecting strong non-random associations of alleles, largely at four loci. Each of the four ETs of *E. coli* that have been most frequently recovered from natural populations has an allozyme profile that is very similar to, or identical with, the hypothetical modal ET of one of the groups. ETs of *Shigella* fall into two of the groups. No biological significance can at present be attributed to the genetic structure revealed by multilocus electrophoretic techniques. The electrophoretic data are fully compatible with other molecular and more conventional evidence of a close affinity between *E. coli* and *Shigella*, and they raise questions regarding the present assignments of certain strains to species. In support of evidence from DNA reassociation tests and serotyping, the present study suggests that *S. sonnei* is homogeneous in chromosomal genotype.

INTRODUCTION

Numerous techniques have been employed to detect genetically determined variation in *Escherichia coli* and related forms of bacteria in an effort to establish criteria for classifying and identifying strains in genetic, epidemiological, and ecological research. Serotyping has revealed more than 1000 distinct combinations of antigens of the O, K, and H groups in isolates of *E. coli* (Eveland *et al.*, 1971; Ørskov *et al.*, 1976, 1977; Bettelheim, 1978); and analysis of chromosomal DNA has shown that pairs of strains may differ by as much as 4% in G + C content, 23% in genome size, and 25% in nucleotide sequence, as indexed by reassociation experiments (Brenner

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Abbreviation: ET, electrophoretic type.

et al., 1969, 1972*a, b*; Brenner & Falkow, 1971; Brenner, 1981). Variation in nucleotide sequence also has been measured by comparing restriction endonuclease fragments (Anilionis & Riley, 1980; Harshman & Riley, 1980) and by sequencing certain regions of the genome (Hori & Osawa, 1979; Milkman & Crawford, 1983). Other methods of detecting genetic diversity include metabolic typing (biotyping) (Krieg & Lockhart, 1966; Colwell *et al.*, 1974; Johnson *et al.*, 1975; Sakazaki *et al.*, 1976; Crichton & Old, 1979, 1982); bacteriocin typing (Gillies, 1978); phage typing (Milch, 1978); testing sensitivity to various chemicals ('resistotyping') (Elek *et al.*, 1973; Old *et al.*, 1980, 1981); electrophoretic analysis of proteins of pili (Jann *et al.*, 1981), of the outer membrane (Achtman *et al.*, 1983), and of whole cells (Bowman *et al.*, 1967; Baptist *et al.*, 1969, 1975; Goullet, 1973, 1980); and the characterization of plasmids (Jamieson *et al.*, 1979; Williams & Warner, 1980; Sansonetti *et al.*, 1981, 1982; review in Elwell & Shipley, 1980).

Although these methods have revealed extensive variation in *E. coli* and related forms, they have not provided data suitable for answering questions concerning even the most basic aspects of population genetics (Jacquard, 1974; Lewontin, 1974). Among these are the following: (1) What proportion of the genetic loci is polymorphic in a population or species? (2) What are the numbers and frequencies of alleles at the average locus in the genome, and what is the variance of genic (allelic) diversity among loci? (3) How do allele frequencies vary temporally within populations and geographically among populations? (4) To what extent can the genotypic arrays of strains in populations (i.e. the combinations of alleles over loci) be predicted from the allele frequencies at individual loci?

To address these questions, protein gel electrophoresis may be used to measure allelic variation at many loci by screening randomly selected sets of enzymes for allozymes – genetically controlled variants of enzymes differing in net electrostatic charge as a consequence of one or more amino acid substitutions. Although this multilocus approach has long been part of the standard methodology of research on the population genetics of higher organisms (e.g. Lewontin & Hubby, 1966; Lewontin, 1974; Nei, 1975; Ayala, 1976; Harris *et al.*, 1977; Selander & Whittam, 1983), it has not been widely applied to bacteria. Its major strength is that it permits one to estimate allelic variation at individual loci (and an average over all loci studied) and yields measures of genetic distance, which can be interpreted in terms of numbers of nucleotide and amino acid substitutions between different individuals, populations, and taxonomic groups (Nei, 1975).

In application to bacterial populations, the multilocus electrophoretic approach has several advantages over other methods of measuring genetic variation. For example, a particular advantage over serotyping and bacteriocin typing is the fact that genotypic data can be obtained for all isolates – none is untypable. Because most of the enzyme loci thus far examined in *E. coli* are represented in populations by many alleles (Selander & Levin, 1980), the power of the technique to resolve genetic differences between strains is very high; and the chances of similarity between strains over a large number of loci due to convergent evolution are very low.

In this paper, we describe the results of a study of the population genetics and evolutionary relationships of *E. coli* and *Shigella*, in which we have examined electrophoretically demonstrable polymorphism in enzymes encoded by 12 gene loci in isolates derived from many natural sources.

METHODS

Sources of strains. The collection of 1608 isolates of *E. coli* analysed in this study is largely a compilation of material from previous electrophoretic studies of enzyme polymorphism conducted in our laboratory (Selander & Levin, 1980; Caugant *et al.*, 1981, 1983). The collection includes: (1) 550 isolates obtained from a single human host in Massachusetts over a period of 11 months; (2) 17 from 16 infants in a hospital nursery in Massachusetts; (3) 34 from 30 adults and children in Iowa; (4) 55 from 41 individual mammals of 28 species in a zoo and in natural habitats in North America; (5) 1 from a lizard; (6) 2 from well water; (7) 1 each of laboratory strains *E. coli* B and C; (8) 24 of *E. coli* K12, representing 5 major lineages (Bachmann, 1972); (9) 121 from urine specimens of 121 Swedish women with urinary tract infections; (10) 47 from urine specimens of 47 schoolgirls in Sweden having asymptomatic bacteriuria; and (11) 100 from 100 Swedish schoolgirls not having urinary tract infections. We have also included data from an unpublished survey of variation in (12) 457 isolates from 3 families in Massachusetts,

each comprising 2 adults, 2 children, and 2 pets (cats, dogs); and (13) 198 isolates from 2 families in New York state.

Except as noted, isolates were obtained from faecal samples. Methods of isolating and identifying *E. coli* are described by Selander & Levin (1980) and by Caugant *et al.* (1981).

Additionally, we have examined 123 strains of 4 species of *Shigella* from various parts of the world. *Shigella boydii*: 22 strains from 20 patients in various hospitals in Massachusetts and 2 strains from Yugoslavia. *Shigella dysenteriae*: 9 strains from Massachusetts and 1 from Nepal. *Shigella sonnei*: 20 strains derived from a local episode of dysentery in the Holyoke/Chicopee area of Massachusetts (traced to contamination of a swimming pool), and 18 strains from Spain, Portugal, Yugoslavia, Tunisia, India, Hong Kong, and several Greek islands. *Shigella flexneri*: 21 strains from associated hosts involved in an episode of dysentery following a picnic in the Worcester/Shrewsbury area of Massachusetts, and 13 strains of world-wide origin. The strains of *Shigella* were collected between 1976 and 1981. Those from Massachusetts were supplied, through Dr Bruce R. Levin, by Mr Rex Bacchieri, Microbiology Department, Massachusetts State Laboratory, Jamaica Plains, Massachusetts; and those from other areas were furnished by Dr Knut Lincoln, of Göteborg, Sweden.

To compare results obtained by electrophoresis of enzymes with those provided by DNA reassociation tests, we examined 11 strains of *E. coli* and 4 strains of *Shigella* that were earlier analysed by Brenner *et al.* (1972a). The strains of *E. coli* were K12, C600, B (Albany), BB, W3442K, 01A, 02A, 04, and A-D 01 111-69 and 05 3121-63, representing the Alkalescent-Dispar biotype. The strains of *Shigella* were *dysenteriae* 2, *boydii* 1, *flexneri* 21 24570, and *sonnei* (virulent).

Electrophoresis of enzymes. Techniques of horizontal starch-gel electrophoresis and the selective demonstration of specific enzyme activity used in our laboratory are those described by Selander *et al.* (1971), with minor modifications for application to bacteria (Caugant *et al.*, 1981). The 12 enzymes that are common to all the above-cited surveys of enzyme polymorphism are listed in Table 1. In the case of the 15 strains obtained from Brenner, variation in indophenol oxidase, phosphoglucomutase, and glyceraldehyde-3-phosphate dehydrogenase was also studied. The structural genes corresponding to most of the enzymes assayed have been mapped to chromosomal locations (Bachmann & Low, 1980).

Using standard procedures of protein electrophoresis, we directly compared the relative mobilities of alternative forms of each enzyme occurring in strains. These allozymes (electromorphs) were equated with alleles at the individual loci encoding the enzymes and were numbered in order of increasing anodal mobility on gels. In strains lacking activity of a particular enzyme, we scored the alleles as 'null'. In this manner, the combination of alleles at each of the 12 enzyme loci (or 15 in the case of Brenner's strains) was determined for each strain. Following Caugant *et al.* (1981), each distinctive combination of alleles (genotype) was designated as an electrophoretic type (ET) (see examples in Table 3).

RESULTS

Proportion of loci polymorphic and number of alleles. All 12 enzymes were polymorphic in *E. coli*, and the number of alleles ranged from 3 for glucose-6-phosphate dehydrogenase to 23 for β -galactosidase, with a mean of 9.3 per locus (Table 1). In *Shigella*, the mean number of alleles was only 2.9 per locus (range, 1-6), and two of the loci were monomorphic. Some 77% of the allozymes recorded in *Shigella* are electrophoretically indistinguishable from those occurring in *E. coli*.

Number of electrophoretic types. Among the 1731 isolates studied, we identified 302 unique combinations of alleles or ETs, of which 279 represent *E. coli* and 23 represent *Shigella*. The mean numbers of isolates per ET were 5.5 for *E. coli* and 5.6 for *Shigella*.

Distribution of ETs in hosts. There is a striking difference between *E. coli* and *Shigella* in the frequency distribution of number of different hosts from which individual ETs were isolated (Table 2). (An ET isolated two or more times from the same individual host was counted only once.) For *E. coli*, 76% of the ETs were each recovered from only one host; and only 12% were isolated from three or more hosts. [The converse is certainly not the case: the intestinal floras of individual hosts include numerous ETs; Caugant *et al.* (1981) detected 53 ETs in one host over an 11-month period.] In contrast, the ETs of *Shigella* on the average were recovered from many more hosts (5.8 hosts per ET) than were those of *E. coli* (1.7 hosts per ET). This difference reflects in part the infection of closely associated hosts by a single clone in local episodes of dysentery. But this is not the whole explanation, for the 13 strains of *Shigella sonnei* from world-wide sources and the 21 strains from associated hosts in Massachusetts all represent a single ET.

Table 1. *Genetic diversity (H) at 12 enzyme loci in natural populations of E. coli and Shigella*

$H = 1 - \sum x_i^2$, where x_i is the frequency of the i^{th} allele. The number of alleles is indicated in parentheses.

Enzyme	Symbol	EC number	<i>E. coli</i> <i>n</i> = 279	<i>Shigella</i> <i>n</i> = 23	Combined <i>n</i> = 302
Alcohol dehydrogenase	ADH	1.1.1.1	0.729 (7)*	0.163 (3)*	0.682 (8)*
Malate dehydrogenase	MDH	1.1.1.37	0.221 (7)	0.227 (2)	0.223 (8)
Isocitrate dehydrogenase	IDH	1.1.1.41	0.440 (6)*	0.526 (3)	0.450 (7)*
6-Phosphogluconate dehydrogenase	6PG	1.1.1.44	0.715 (20)*	0.627 (6)*	0.739 (20)*
Glucose-6-phosphate dehydrogenase	G6P	1.1.1.49	0.192 (3)	0 (1)	0.178 (3)
Glutamic-oxaloacetic transaminase	GOT	2.6.1.1	0.049 (4)	0.420 (4)	0.084 (5)
Adenylate kinase	AK	2.7.4.3	0.423 (5)	0 (1)	0.399 (5)
β -Galactosidase	β GA	3.2.1.23	0.893 (23)*	0.083 (2)*	0.904 (23)*
Phenylalanyl-leucine peptidase	PE2	3.4.11.-	0.598 (9)*	0.083 (2)	0.569 (9)*
Aconitase	ACO	4.2.1.3	0.616 (9)	0.556 (4)*	0.648 (10)*
Phosphoglucose isomerase	PGI	5.3.1.9	0.594 (10)	0.159 (2)	0.596 (11)
Mannose phosphate isomerase	MPI	5.3.1.8	0.710 (9)*	0.646 (5)	0.729 (11)*

* Includes null phenotype.

Table 2. *Frequency distribution of ETs isolated from different numbers of unassociated hosts*

Each entry is the number of distinct ETs found in the specified number of hosts.

Species	No. of hosts . . .	1	2	3	4	5	6	7	≥8	Ave. no. of hosts per ET
<i>Escherichia coli</i>	211	34	18	1	4	2	4	5		1.69
<i>Shigella</i> spp.	8	0	6	2	3	0	0	4		5.78

Some ETs of *S. boydii* isolated in 1976 in Massachusetts are electrophoretically identical to those recovered following an episode of dysentery in 1981. We do not have extensive information on the temporal stability of *E. coli* genotypes, because most ETs in our collection were obtained from single hosts. However, isolates that are electrophoretically indistinguishable from laboratory strain K12, which was originally obtained from a human host over 50 years ago (Bachmann, 1972), have been repeatedly found in natural populations in the course of our studies and, in fact, represent the fourth most commonly recorded ET of *E. coli*. Moreover, those ETs of *E. coli* that are most frequently recovered are not restricted geographically or in type of host. For example, ET-136 is represented by isolates from human siblings and a dog in Massachusetts and from 11 girls with urinary tract infections and 2 healthy girls in Sweden.

Genetic diversity among electrophoretic types. Estimates of genetic diversity (Nei, 1975), calculated from the frequencies of alleles in the 302 ETs, are presented in Table 1. The mean diversity per locus over all ETs of *E. coli* is 0.52, which is similar to the value of 0.47 reported by Selander & Levin (1980) for 20 enzyme loci assayed in 109 strains from natural populations of humans and a variety of mammals. There is much less genetic diversity in the ETs of *Shigella*, the comparable value being only 0.29.

Genetic distance between E. coli and Shigella. We have calculated two measures of genetic distance between *E. coli* and *Shigella* (Fig. 1). One measure expresses distance as the mean number of loci at which alternative alleles occur (i.e. the number of mismatches) in pairwise comparisons between all ETs in the two taxa. The other measure of distance is Nei's (1975) *D*,

Fig. 1. Two measures of genetic distance between *E. coli* and *Shigella* species and among the three groups of *E. coli*. Left-hand side: the number of loci at which alternative alleles occur in hypothetical modal ETs of *E. coli* and *Shigella*. Right-hand side: Nei's genetic distance (D), calculated from allele frequencies within each taxon or group.

which, with certain assumptions, can be interpreted in terms of mean net amino acid differences per polypeptide or codon differences per locus between strains. Our analysis yields a mean estimate of about 0.3 detectable codon differences per locus between pairs of strains of *E. coli* and *Shigella*. On the assumption that electrophoresis detects about 25% of all amino acid substitutions (which probably is a conservative estimate: Bonhomme & Selander, 1978; Ramshaw *et al.*, 1979; Ayala, 1982), this result translates to about 1.2 amino acid differences for the average enzyme.

Genetic structure of populations. To visualize patterns of genetic structure, as reflected by similarity of allozyme profiles among ETs, we used principal components analysis (Sneath & Sokal, 1973). In preparation for this analysis, we converted the electrophoretic data into a binary code by considering each allele as a variable that can occur in one of two states, either present (1) or absent (0), in a strain; and the total number of variables is simply the sum of the alleles over all loci. This method of coding is sensitive to single allelic substitutions between strains and can accommodate null phenotypes.

The results of the analysis are shown in Fig. 2, which is a plot of the scores for all ETs on the first two principal axes (factors). These axes explain about 10% of the total variance among ETs, which suggests that there are strong correlations in the occurrence of particular allozyme combinations in ETs.

The projection of ETs on the first two axes reveals that they fall into three overlapping clusters. From each of the three clusters, we chose a random set of 20 ETs and, by means of a discriminate function analysis, classified each of the remaining ETs into one of these three groups (see Fig. 2). For the discriminate analysis, the electrophoretic data were coded by translating the consecutive numbers representing allozyme mobilities of an enzyme on to a common scale by ranging (Sneath & Sokal, 1973). By standardizing the allelic variation at each locus into the range 0 to 1, this method weights all loci equally, even though the number of allozymes varies over loci. It also retains information about the relative mobility of different allozymes; but because null alleles could not be coded, they were classified with the most common allozyme allele at a locus.

Fig. 2. Plot of scores of electrophoretic types (ETs) of *E. coli* ($n = 279$) and *Shigella* ($n = 23$) for the first two principal factors. Electrophoretic types of Groups I, II, and III of *E. coli* are designated by ○, ×, and ●, respectively. S indicates *Shigella*. (From Whittam *et al.*, 1983, with permission of the authors.)

Table 3. *Comparison of modal and most common ETs in three groups of E. coli*

Entries are allozyme designations for the three modal ETs and the four most frequently recovered ETs. The number of unassociated host individuals from which each ET was recovered is indicated in parentheses. The allozyme designations that differ in the modal and the most common ETs of a group are shown in bold type.

Enzyme	Group I		Group II		Group III		
	Modal ET	ET-4 (12)	Modal ET	ET-99 (17)	Modal ET	ET-136 (16)	ET-58 (28)
ADH	1	1	6	6	6	6	6
MDH	2	2	2	6	2	2	2
IDH	2	2	5	5	5	5	5
6PG	6	6	6	4	6	6	6
G6P	2	2	2	2	2	2	2
GOT	3	3	3	3	3	3	3
AK	4	4	4	5	5	4	5
βGA	15	15	15	7	7	7	7
PE2	5	5	5	5	4	4	2
ACO	7	7	6	7	6	6	6
PGI	4	4	7	7	7	7	7
MPI	3	3	7	7	3	3	3

Within *E. coli*, the average genetic distance between strains in Group I and those in Groups II and III ($D = 0.41$) is about 30% greater than that between *E. coli* as a whole and *Shigella* ($D = 0.30$) (Fig. 1). Groups II and III, however, are more closely related, with a genetic distance of about 0.20 detectable codon differences per locus.

For each of the three groups, we constructed the hypothetical modal ET, which is the combination of the most common allele at each of the 12 enzyme loci within a particular subdivision. (Note that because the genotypes of the modal types were calculated from the allele frequencies of the ETs in the clusters, rather than from the total numbers of isolates, they are not influenced by the frequencies of isolation of the various ETs.) The four ETs of *E. coli* that have

Fig. 3. Dendrogram of the 23 electrophoretic types of *Shigella*, produced by average linkage clustering. [D, which is equal to one minus Jaccard's similarity coefficient (Sneath & Sokal, 1973), is given as the percentage of the maximum value.]

been most frequently recovered (isolated from 10 or more hosts) are distributed in all three groups, and each has an allozyme profile that is very similar, or in one case identical, to the modal ET of a cluster (Table 3). The modal ETs differ at 5, 4, and 7 loci for the comparisons of *E. coli* Groups I and II, *E. coli* Groups II and III, and *E. coli* Groups I and III, respectively. It is noteworthy that the genotypic combination of the commonest alleles in the sample of all 302 ETs has never been recovered.

Variation within *Shigella*. A dendrogram of the 23 ETs of *Shigella* in our sample, produced by average linkage clustering (Sneath & Sokal, 1973), reveals two major clusters (Fig. 3). Cluster A consists largely of ETs of *flexneri* but also includes ETs representing certain strains of *boydii* and *dysenteriae*. Cluster B contains ETs of all four species; and *sonnei* appears to be the most distantly related form. ET-2, which is represented by two isolates of *boydii* of the same serotype from an individual in Massachusetts, is quite distinct, joining clusters A and B at the 40% level of similarity.

DISCUSSION

An assumption underlying many of the applications of the multilocus electrophoretic technique to population genetics and systematics is that the loci encoding the enzymes analysed are a representative sample of the genome (Lewontin & Hubby, 1966; Lewontin, 1974). If this is indeed the case, one would expect to find a positive correlation between estimates of relatedness among a group of strains obtained by enzyme electrophoresis and those derived from DNA hybridization, which measures similarity in total nucleotide sequence. Such a relationship has been demonstrated in higher organisms (Wilson *et al.*, 1977) and is also apparent in the case of *E. coli* and *Shigella* (Table 4). For strains that earlier were analysed by DNA hybridization by Brenner *et al.* (1972*a*, 1973), Table 4 shows separately estimates of genetic divergence from *E. coli* strains K12 and BB as standards. For both comparisons, there are significant positive

Table 4. *Relationships between estimates of genetic divergence obtained by electrophoresis of 15 enzymes and by DNA reassociation experiments*

Strain	Compared with <i>E. coli</i> K12		Compared with <i>E. coli</i> BB	
	No. of allozyme differences	ΔT_m at 75 °C*	No. of allozyme differences	ΔT_m at 75 °C*
<i>E. coli</i> K12	0†	—	3	0.2
C600	2	0.3	4	0.5
B	5	1.0	6	NM
BB	3	0.6	0†	—
B (Albany)	5	0.4	6	0.6
N3442K	4	1.0	1	0.4
01A	9	2.8	9	2.7
02A	12	1.7	13	1.7
04	12	2.0	13	1.7
A-D01	6	1.2	7	1.7
A-D05	3	1.3	5	0.9
<i>Shigella boydii</i> 1	3	NM	3	NM
<i>S. flexneri</i>	6	0.8	7	1.0
<i>S. sonnei</i> (virulent)	4	0.7	3	NM
<i>S. dysenteriae</i> 2	7	0.5	8	1.2
Correlation (<i>r</i>)	0.650 (<i>P</i> < 0.05) <i>n</i> = 13		0.745 (<i>P</i> < 0.01) <i>n</i> = 11	

NM, Not measured.

* ΔT_m values from Brenner *et al.* (1972a).

† Standard.

correlations between the percentage divergence in nucleotide sequences estimated by the decrease in thermal elution midpoint (ΔT_m) at 75 °C and the number of allozyme differences at enzyme loci.

Our analysis of electrophoretically demonstrable polymorphism in 12 enzymes has revealed extensive genetic structure in natural populations of *E. coli*. Because high rates of recombination between loci in the absence of natural selection for specific combinations of alleles (epistatic selection) would tend to prevent the generation and maintenance of genetic structure, it is important to note that, contrary to the suggestion by Milkman (1973, 1975), available evidence indicates that recombination occurs very infrequently in natural populations (Selander & Levin, 1980; Caugant *et al.*, 1981; Achtman *et al.*, 1983). With low rates of recombination, natural populations are mixtures of more or less independently evolving lines (clones); and we would expect non-random associations of alleles at different loci, even in the absence of epistatic selection (Kubitscheck, 1974; Koch, 1974, 1981; Maynard Smith & Haigh, 1974; Thomson, 1977; Ohta, 1982). Elsewhere, we (Whittam *et al.*, 1983) have tested deviations from independent assortment of alleles over loci in *E. coli* by comparing the distribution of the number of allozyme differences between ETs in all pairwise comparisons to an hypothetical one based on allele frequencies in all ETs (Brown *et al.*, 1980). The variances of the observed and expected distributions differed significantly, indicating a strong degree of multilocus association (i.e. linkage disequilibrium) among loci. We have also shown that significant allele associations at four loci are largely responsible for the clustering of ETs into three groups.

We cannot at present attribute any biological significance to the subspecific genetic structure in *E. coli* revealed by our analysis or account for maintenance of the three clusters of genotypes in the face of mutation and recombination, even at very low rates. In an attempt to obtain clues to possible biological correlates of polymorphic enzyme variation, we cross-referenced each ET of *E. coli* with certain attributes of its source population, and, when sample sizes were sufficient for comparison, performed association tests. With respect to host type (human vs. non-human), diet of host (carnivorous vs. herbivorous), and association with urinary tract infections (ETs isolated

from urine of women with such infections vs. ETs from faeces of healthy persons), we have found no significant group-character associations. However, there is a geographic component to the distribution of ETs, as shown by the fact that among 30 human hosts in Massachusetts, including individuals in three families and a group of newborn infants, ETs in Groups I and II are disproportionately represented. An analysis of within- and between-region components of the total variance in linkage disequilibrium in *E. coli* will be presented elsewhere.

The results of our multilocus electrophoretic study are compatible with those of other recent work on variation at the molecular level, which have confirmed earlier suggestions, based on metabolic typing, serotyping, and other more conventional characters (Edwards & Ewing, 1972; Johnson *et al.*, 1975; Rowe *et al.*, 1976; Sanderson, 1976), of a close affinity between *Shigella* and *E. coli*. The molecular evidence comes from a large variety of sources, including studies of DNA reassociation (Brenner & Falkow, 1971; Brenner *et al.*, 1972a); nucleotide sequences of the *ompA* (Braun & Cole, 1982) and *trp* regions (Nichols *et al.*, 1980); complementarity in tRNAs and 5S rRNAs (Brenner *et al.*, 1977); copy number of the IS1 insertion element (Nyman *et al.*, 1981); sequence of ribosomal proteins (Hori & Osawa, 1978); the N-terminal amino acid sequence of the tryptophan synthetase α subunit (Li & Hoch, 1974); immunoelectrophoresis of β -lactamase (Bergstrom *et al.*, 1982); and electrophoresis of enzymes (Bowman *et al.*, 1967; Baptist *et al.*, 1969; Goullet, 1980). Although most of these studies compared relatively small numbers of strains and therefore did not adequately determine the extent of genetic variation within species in relation to that between species, the cumulative evidence demonstrates that *Shigella* and *E. coli* are sufficiently similar to warrant them being placed in the same genus. Justification for the continued use of conventional nomenclature for these bacteria is based entirely on historical and clinical considerations (Brenner *et al.*, 1973; Ørskov *et al.*, 1977).

The dendrogram of ETs in Fig. 3 only loosely corresponds to the current classification of *Shigella* into four species. Particularly significant is the observation that, in several cases, the same ET is represented by isolates received as *boydii* and *flexneri* or as *boydii* and *dysenteriae*. The classification of certain strains of *Shigella* has been questioned (Johnson *et al.*, 1975; Petrovskaya & Bondarenko, 1977; Petrovskaya & Khomenko, 1979), and the electrophoretic evidence presented here and elsewhere (Goullet, 1980) clearly indicates the need for taxonomic revision. Multivariate analyses of as many as 200 phenotypic characters have produced a variety of schemes of taxonomic relationship among the four species, but, in general, *dysenteriae*, *boydii*, and *flexneri* show greater overall similarity to one another than to *sonnei* (Colwell *et al.*, 1974; Johnson *et al.*, 1975; Dodd & Jones, 1982). As shown in Fig. 3, our analysis demonstrates a similar pattern of relationships.

The fact that the studies of Brenner *et al.* (1973) on DNA reassociation detected marked divergence between the *boydii* 13 strain and all other strains of *Shigella* suggests that *boydii* is genetically more heterogeneous than the other species; and our results support this hypothesis. Note particularly that one strain of *boydii* is markedly different from all other strains of *Shigella* that we studied.

The evidence from DNA reassociation tests (Brenner *et al.*, 1973), serotyping (Ørskov *et al.*, 1977), and our study of allozyme variation suggests that all strains of *Shigella sonnei* are genetically homogeneous, insofar as the chromosomal genotype is concerned. A relatively limited amount of variation in metabolic characters (Szturm-Rubinsten, 1964), sensitivity to certain toxic chemicals (Helgason & Old, 1981; Old *et al.*, 1981), and some other phenotypic characters (Dodd & Jones, 1982) has been detected, together with some minor variation in esterase zymograms associated with biotype (Goullet, 1980), but the extent to which these variations represent *in vitro* mutation (see discussion in Helgason & Old, 1981) or are mediated by plasmids is unknown. It has recently been shown that resistance to several antibiotics, bacteriocin production, and expression of the form I somatic antigen, all of which characters have been used in epidemiological studies, are controlled by factors carried by plasmids (Jamieson *et al.*, 1979; Kopecko *et al.*, 1980; Sansonetti *et al.*, 1981).

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