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Genetics

Distribution of DNA insertion element IS*5* in natural isolates of *Escherichia coli*

(transposable elements/genetic divergence/electrophoretic types)

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ABSTRACT **DNA from *Escherichia coli* strains in a reference collection of 72 recent natural isolates (ECOR strains) and 25 natural isolates from the "pre-antibiotic" period 1930–1940 (Murray strains) were studied to determine the genomic abundance of insertion element IS*5* and the size of genomic restriction fragments carrying sequences homologous to IS*5*. Among the ECOR strains, nearly two-thirds lack DNA sequences that hybridize with IS*5*, and one-half of the remainder have only one copy. Among strains in which IS*5* is present, extensive variation in the size of IS*5*-bearing restriction fragments occurs, in many cases allowing distinction among strains that are judged to be nearly identical in genotype because of the identical electrophoretic mobility of the enzyme coded by each of 11 chromosomal loci. Among the Murray strains in which IS*5* is present, the average number of elements per strain is larger, but not markedly so, than among recent isolates. Comparison between duplicate strains in the Murray collection suggests that the rate of accumulation of IS*5* elements in prolonged storage in stab tubes corresponds to an apparent probability of transposition of ≈0.008 ± 0.002 per IS*5* element per year. Because of the extensive genetic variation among strains, insertion elements such as IS*5* would seem to be convenient genetic markers with which to detect recent common ancestry among strains.**

Insertion sequences (IS) are prokaryotic DNA elements that are capable of transposition into several or many sites within the genome (1, 2). Such elements, which range in size from 800 to 2000 base pairs, are ubiquitous in prokaryotes and in their bacteriophages and plasmids. They evidently carry little or no genetic information other than that required for the occurrence and regulation of transposition. Although much is known about the DNA sequence organization and the molecular biology of insertion sequences, little is known about the mechanisms governing their persistence and abundance in natural populations (3, 4). Documented genetic effects of IS sequences include the fusion of independent replicons, formation of deletions near the site of insertion, and altered expression of nearby genes owing to transcriptional start or stop signals present in the elements (5–8). In at least one case, an IS interacts physiologically with the host, resulting in an increased growth rate of host cells under particular environmental conditions (9).

The genome of *Escherichia coli* K-12 carries from 1 to 10 copies of at least five distinct IS, designated IS*1*–IS*5* (1). Studies of several IS elements among a limited number of laboratory strains and natural isolates indicate that the number of copies of a particular element can vary widely (10–12). Riley and co-workers have also found extensive variation among natural isolates in the size of restriction fragments carrying sequences homologous to bacteriophage λ and in the number of such fragments (13, 14). The present report concerns the distribution of IS*5* among 97 natural isolates of *E. coli*. These 97 strains represent two collections. One, the ECOR collection, is a reference collection of 72 strains representing a diversity of genotypes as assessed by electrophoretic enzyme variation (15). Most of the ECOR strains were isolated within the past 10 years. The remaining 25 strains are from the Murray collection, a diverse assemblage of Enterobacteriaceae collected by E. D. G. Murray between the years 1917 and 1954 (16). The Murray strains of *E. coli* included in the present study were isolated 45–55 years ago. We were interested in the distribution of IS*5* among a diverse sample of recent isolates of *E. coli* and in the dynamics of accumulation of IS*5* in bacterial cultures undergoing prolonged storage in stab tubes. IS*5* was chosen because it is the most abundant insertion sequence known to be present in the genome of *E. coli* K-12.

IS*5* is 1195 base pairs long (17, 18). It codes for two proteins: a large one with a coding region of 978 base pairs extending through most of the element, and a small one with a coding region of 324 base pairs completely included within the other but transcribed from the opposite strand (19, 20). The ends of IS*5* form an imperfect 16-base-pair inverted repeat (one mismatch), and the element creates a 4-base-pair duplication of target sequence at the site of insertion. The consensus target sequence of IS*5* insertion is C-(A or T)-A-(A or G) (17).

Among the ECOR and Murray strains, we find extensive genetic variation in the number of copies of IS*5*, including many strains that lack the element, as well as in the size of DNA restriction fragments that contain sequences homologous to IS*5*. Genetic similarity with respect to IS*5* is retained only among strains that seem to have diverged very recently. We suggest that IS*5* and other insertion sequences will be useful genetic markers in the study of closely related strains.

MATERIALS AND METHODS

Strains. The ECOR collection of *E. coli* strains was generously provided by H. Ochman and R. K. Selander. The strains are numbered sequentially from 1 through 72. Details on the sources of the strains and their electrophoretic type at 11 enzyme loci are provided by Ochman and Selander (15). The 25 *E. coli* strains in the Murray collection were kindly provided by N. Datta. Details concerning the Murray strains are provided by Hughes and Datta (16). The numbers of the Murray strains are those assigned by the National Collection of Type Cultures (London). In this paper, the strain numbers are preceded by an M to indicate that the strains derive from the Murray collection.

An *E. coli* strain carrying plasmid pMN4 was kindly provided by R. E. Wolf, Jr., for the isolation of an IS*5*-specific hybridization probe. The plasmid consists of pBR322 with an *E. coli gnd*-bearing *Eco*RI restriction fragment inserted into the *Eco*RI site. This *E. coli* fragment also carries most of the

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Abbreviations: IS, insertion sequence(s); kbp, kilobase pairs.

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IS*5* element normally found upstream of the *gnd* gene (clockwise from *gnd* in the genetic map) in *E. coli* K-12 (21, 22). A detailed restriction map of pMN4 is provided by Nasoff and Wolf (23).

DNA Hybridization. Methods of DNA extraction, digestion with restriction endonuclease, gel electrophoresis, transfer to nitrocellulose filters, and hybridization with radioactive probe have been described (9). In the present experiments, DNA extracted from natural isolates was digested with *Eco*RI, and after gel electrophoresis and transfer to nitrocellulose, it was hybridized with the IS*5*-specific probe described below.

To identify strains lacking IS*5* elements, all strains were initially screened by means of dot blots. For this purpose, overnight cultures of 0.4 ml were centrifuged and resuspended in 0.2 ml of 50 mM Tris·HCl/50 mM EDTA, pH 8.0. Cells were incubated at 37°C for 15–30 min after addition of 0.2 ml of protease K (5 mg/ml), and for an additional 15 min after addition of 10% (wt/vol) sodium lauryl sulfate. Suspensions were chilled on ice, and after addition of 22 μl of 5 M NaOH, were incubated at room temperature for 15–30 min. Suspensions were again chilled, and 140 μl of 3 M NH_4Ac was added. Resulting samples were placed in the wells of a Schleicher & Schuell Micro-Sample Filtration Manifold apparatus containing Whatman 3MM filter paper supporting a Schleicher & Schuell BA85 nitrocellulose filter previously soaked in water and then in 1 M NH_4Ac. After the filtration of samples through the nitrocellulose, the filter was dried in air, baked at 80°C for 2 hr, and hybridized with probe as described (9).

Probe. Fig. 1 is an abbreviated restriction map of plasmid pMN4. The bar corresponds to the inserted *E. coli Eco*RI fragment, and the thin lines correspond to sequences in pBR322. The location of part of IS*5* in the plasmid is indicated. The *Eco*RI site comprises nucleotides 99–104 (numbering from the left), and the *Bgl* II site comprises nucleotides 1038–1043, of the 1195-base-pair IS*5* element (17). Purified plasmid was cleaved with *Eco*RI and *Bgl* II, and the resulting 0.94-kilobase-pair (kbp) *Eco*RI/*Bgl* II restriction fragment was isolated from agarose by means of the glass powder method (9). This fragment was used as the IS*5*-specific probe in all DNA hybridizations.

RESULTS

The overall distribution of IS*5* among ECOR and Murray strains is summarized in Table 1 and Fig. 2. The three groups of strains in the ECOR collection are those defined by Whittam *et al.* (24) and by Ochman and Selander (15) as representing three major subdivisions of natural isolates of *E. coli* based on the electrophoretic type of allele present at 11 chromosomal loci.

The data in Table 1 and Fig. 2 were obtained in two series of experiments. First, all strains were screened for the presence of IS*5* by means of dot hybridizations. This procedure served to identify the strains lacking DNA sequences homologous to IS*5*. Dot hybridization data for the ECOR strains are shown in Fig. 3. Control hybridizations (data shown below) with restriction enzyme digests of DNA from these strains, including a subset lacking IS*5*, indicate that the number of copies of IS*5* present in a strain cannot be estimated reliably by the dot blot method, particularly when the number of copies is more than a few. However, strains lacking IS*5* can be identified unambiguously.

Table 1. Occurrence of IS*5* in natural isolates

Strains	No. lacking IS*5*	No. with IS*5*	Mean copies of IS*5* ± SEM (excludes 0 class)
ECOR group I	13	13	3.2 ± 0.8
ECOR group II	16	8	2.0 ± 0.4
ECOR group III	18	4	2.2 ± 1.2
Murray	9	16	5.7 ± 1.2

All strains containing IS*5* identified in the dot blots were studied further in IS*5*-probed Southern blots (25) of bacterial DNA digested with the restriction enzyme *Eco*RI. An example is shown in Fig. 4. In such an experiment, each IS*5* in the genome should theoretically produce a single hybridizing DNA fragment with one *Eco*RI cleavage site within IS*5* itself and the other *Eco*RI cleavage site in the host DNA to the right of the element as it is oriented in Fig. 1. Two IS*5* elements in opposite orientation not flanking a host *Eco*RI site would also produce a single band, but it would be expected to have approximately double the intensity of bands containing only a single copy of IS*5*. These cases cannot be distinguished from those resulting from the superposition of two fragments of nearly the same molecular weight, each containing a single IS*5*. However, owing to the small number of IS*5* elements found in most of these strains, examples of bands with apparent double intensity are infrequent. The relatively small number of IS*5* elements usually results in good separation of IS*5*-hybridizing bands that can be counted without ambiguity. Very strong hybridization signals indicative of IS*5* elements present in high-copy-number plasmids were not observed. Elements present in low-copy-number plasmids cannot be distinguished from chromosomally borne elements using our procedures.

Five strains among the ECOR collection exhibited one or more exceptional restriction fragments that hybridized

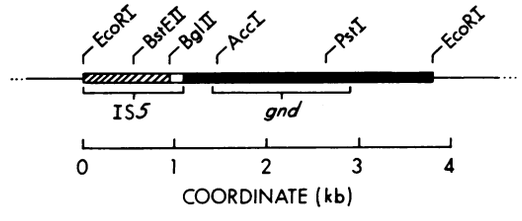

FIG. 1. Partial restriction map of *E. coli* DNA in pMN4 showing position of cloned region of IS*5* and the shaded *Eco*RI/*Bgl* II fragment used as probe. Thin lines represent pBR322 sequences, *gnd* represents the gene for glucose-6-phosphate dehydrogenase.

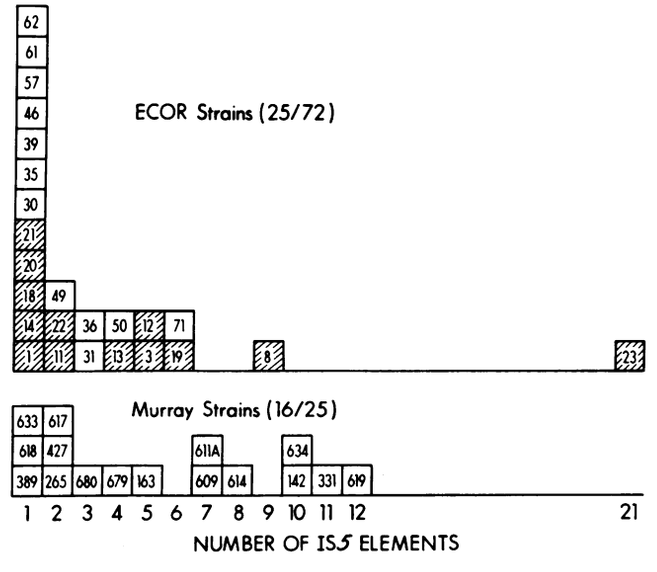

FIG. 2. Distribution of number of copies of IS*5*-hybridizing sequences among 25 ECOR and 16 Murray strains. Strains lacking IS*5* not shown. Numbers correspond to strain numbers in the two collections, and shaded squares correspond to ECOR strains in group I.

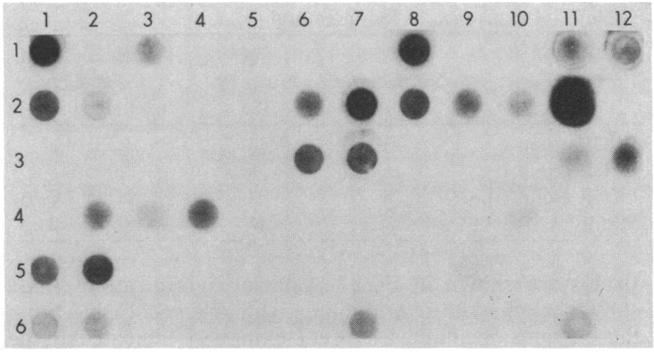

FIG. 3. Dot hybridizations showing relative abundance of IS5 among ECOR strains. The ECOR strain number corresponding to row i and column j is 12(i − 1) + j. Control Southern blots confirm that strains with no hybridization lack IS5 and strains with the weakest positive signals have a single copy of IS5.

weakly with IS5, in addition to having one or more restriction fragments that hybridized normally. These exceptional fragments evidently carry a DNA sequence sharing some homology with IS5, and the normal IS5 sequences in the same strains serve as internal controls. Strains 18 and 35 each had one weakly hybridizing restriction fragment, strains 23 and 31 each had two, and strain 1 was exceptional in having 15. Such IS-related elements are found frequently in similar studies. Nyman *et al.* (12) report 3 among 12 natural isolates containing IS*1*-related sequences, and Schoner and Schoner (11) report IS5-related sequences in *Bacillus*, *Pseudomonas*, and *Serratia*. Possibly, the weakly hybridizing IS5-related sequences reported in these species may be similar, if not identical, to the IS5-related sequences we have found in *E. coli*. In any case, the weakly hybridizing elements are not included in the data in Table 1 and Fig. 2.

Number of Strains Lacking IS5. Comparison of the number of strains lacking IS5 among the four subsets of strains was carried out by means of χ^2 tests using the data in Table 1

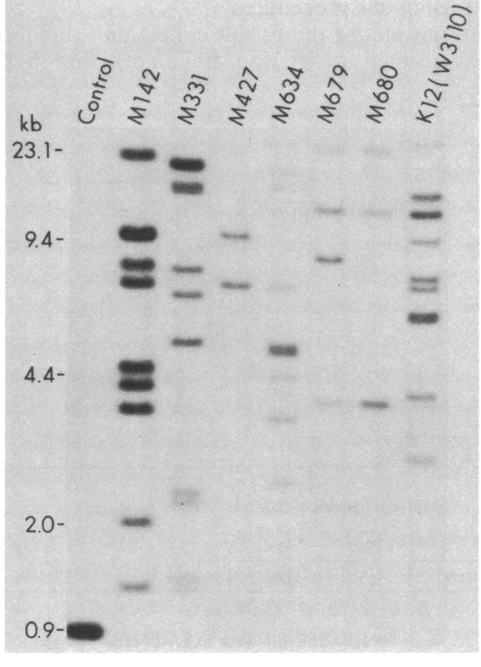

FIG. 4. Southern hybridization of *Eco*RI-digested DNA from several Murray strains and *E. coli* K-12. Control lane contains an *Eco*RI/*Bgl* II double digest of pMN4. Note the similarity between strains M679 and M680.

after elimination of duplicates. In the ECOR collection, four pairs of strains (numbers 20–21, 35–36, 49–50, and 61–62) have recent common ancestry, as judged by their identical electrophoretic phenotype and one or more shared IS5-bearing restriction fragments. Among the Murray strains, three pairs of strains (numbers M389–M617, M609–M611A, and M679–M680) are apparent duplicates according to Murray's collection records, which is confirmed by obvious similarities in their pattern of IS5-bearing restriction fragments (see below). In the statistical analysis, one of each of these duplicate pairs of strains was eliminated to ensure statistical independence of the isolates.

Among the ECOR strains, the excess of IS5-bearing strains in group I is statistically significant ($P = 0.015$). Similarly, the Murray collection has a significant excess of IS5-bearing strains as compared with ECOR groups II and III ($P = 0.002$), but the difference between the Murray strains and ECOR group I is not statistically significant. Consequently, based on the presence or absence of IS5-hybridizing sequences, the strains in Table 1 appear to separate into two classes—one consisting of ECOR groups II and III, and the other consisting of ECOR group I and the Murray strains. Since group I is the most common group among *E. coli* isolates (24), it is not surprising that the Murray collection should resemble group I more than the others.

Distribution of Number of Copies of IS5. The number of IS5 elements present in ECOR and Murray strains is illustrated in Fig. 2. The group I ECOR strains are set off by shading. The average number of IS5 elements per strain, among those having at least one, is shown in Table 1. Significance of differences among the four groups of strains was assessed by analysis of variance. Although the difference in mean number of copies among the four groups in Table 1 is statistically significant ($P < 0.05$), ≈80% of the variation in copy number among strains is attributable to variation within groups. The fraction of the total variance in copy number attributable to variation among groups is only 20%.

The average number of IS5 elements among the Murray strains is slightly, but significantly, greater than the average number among the ECOR group I strains ($P = 0.05$). However, the ECOR groups do not differ significantly among themselves in terms of average number.

Genetic Divergence Between Strains. Certain pairs of strains in the ECOR collection have indistinguishable electrophoretic phenotypes for the enzyme at each of the 11 diagnostic loci. The electrophoretic identity of these strains presumably reflects common ancestry, and the strains therefore permit an explicit test of the hypothesis that the number and distribution of IS within the genome changes more rapidly than conventional genetic markers. The strains in question are shown in Table 2, with each subgroup of strains representing an electrophoretically identical set. The braces indicate identical molecular weight of IS5-bearing fragments. For example, with strains 35 and 36, the brace means that one of the three IS5-bearing bands in strain 36 is identical to the one in strain 35. In strains 49 and 50, two of the four bands in strain 50 are identical to those observed in strain 49. The ECOR collection contains five additional groups of 2–5 electrophoretically indistinguishable strains. The strains in these groups are not listed in Table 2 because all of them lack IS5.

As Table 2 indicates, electrophoretically identical strains can usually be readily distinguished by the number or molecular weight of IS5-bearing fragments. Indeed, strains can usually be distinguished by the number of IS5-bearing fragments, so possible differences in fragment size resulting from genetic variation in the location of host restriction sites does not create significant ambiguity. In only one comparison, between strains 14 and 18, could the difference between the strains possibly result solely from a difference in the lo-

Table 2. Divergence between electrophoretically identical strains

ECOR no.	Source	IS5, no.
1 (RM74A)	Human female (Iowa)	1
2 (STM1)	Human male (Iowa)	0
3 (WIR1)	Dog (Massachusetts)	5
8 (RM77C)	Human female (Iowa)	9
9 (FN98)	Human female (Sweden)	0
10 (ANI)	Human female (New York)	0
11 (C97)	Human female (Sweden)	5
13 (FN10)	Human female (Sweden)	4
14 (P62)	Human female (Sweden)	1
18 (RM210F)	Celebese ape (Washington zoo)	1
20 (RM213I)	Steer (Bali)	1
21 (RM213K)	Steer (Bali)	1
29 (RM3A)	Kangaroo rat (Nevada)	0
30 (RM10A)	Bison (Alberta)	1
31 (RM12)	Leopard (Washington zoo)	3
43 (FN36)	Human female (Sweden)	0
35 (RM42B)	Human male (Iowa)	1
36 (RM77B)	Human female (Iowa)	3
38 (RM75A)	Human female (Iowa)	0
39 (FN104)	Human female (Sweden)	1
40 (P60)	Human female (Sweden)	0
41 (T44)	Human male (Tonga)	0
49 (FN90)	Human female (Sweden)	2
50 (P97)	Human female (Sweden)	4
61 (FN23)	Human female (Sweden)	1
62 (P69)	Human female (Sweden)	1

Braces indicate IS5-bearing fragments of identical molecular weight.

cation of a host restriction site.

Among the 10 groups of strains having identical electrophoretic types, only 2 groups were identical with regard to IS5. The identical size of IS5-hybridizing fragments in strains 20 and 21 was unexpected in light of the previous finding that strain 20 carries more than 30 copies of IS1, whereas strain 21 carries none (12). Strains 61 and 62 were both isolated from the same population of Swedish schoolgirls. Altogether, Table 2 contains 10 groups of electrophoretically identical strains, of which 8 have obvious heterogeneity in their number of copies of IS5. Some of these differences could conceivably result from the presence or absence of IS5-bearing plasmids. In any case, the pattern of IS5-hybridizing fragments among strains does change rapidly as compared with electrophoretic type, both in the number of insertion elements and in their distribution within the genome. Studies of such elements may therefore provide a method for the detection of very recent common ancestry among strains.

Apparent Rate of Transposition. Three pairs of strains in the Murray collection provide a means of estimating the apparent rate of transposition of IS5 in cultures stored for prolonged periods in stab tubes. In two cases, Murray isolated the pair of strains from the same organism and assigned the same strain number, but the members of the pair differ in growth habit. Strains M389 and M617 were isolated separately because the latter produces mucoid colonies. Strains M609 and M611A were isolated separately because they have different colony size. Strains M679 and M680 derive from large and small colonies isolated from a single Murray culture when it was opened in 1980. In Southern blots probed with IS5, the common ancestry of each pair of strains is obvious inasmuch as each pair shares at least one common IS5-bearing restriction fragment. Presumably, the phenotypic differences between the strains are unrelated to their number or distribution of IS5.

Comparisons of IS5 in the apparent duplicate isolates in the Murray collection are shown in Table 3. The number of IS5 elements differing between the strains may be used to estimate the apparent rate of transposition occurring since the storage tubes were established. The estimate is an apparent rate of transposition rather than an actual rate, because it invokes two major assumptions: (*i*) that the members of each pair were identical with respect to IS5 when the storage tubes were initially established and (*ii*) that each element that is different between the members of a pair represents a new transposition event (as opposed to, for example, the deletion of an element or the occurrence of a chromosomal inversion or mutation affecting the distance between IS5-flanking restriction sites). Relative to strains M679 and M680, for example, three common fragments in each of the two strains represents a total of six elements present in storage for 49 yr. The apparent number of transposition events (IS5-bearing fragments different between the strains) is 1. The apparent transposition rate is therefore $1/(6 \times 49) = 0.0034$ per IS5 element per yr. Estimates for the other two pairs of strains are obtained similarly. The average apparent rate estimated from Table 3 is 0.008 ± 0.002 per IS5 element per yr, where the standard error is the standard error of the mean.

DISCUSSION

More than two-thirds of the ECOR strains lack DNA sequences that hybridize with IS5. In this respect, laboratory strains are unrepresentative of natural isolates. Among the laboratory strains K-12, C, W, and B, only strain B lacks IS5 (11). Whether the absence of IS5 in many strains is biologically significant is unclear. The simplest hypothesis is that strains lacking IS5 have simply escaped infection by the element during their lineage. This hypothesis is consistent with the finding (Table 2) that strains that are identical in electrophoretic phenotype may nevertheless differ in the presence or absence of IS5. However, we cannot exclude the possibility that genetic differences among strains may affect the maintenance or rate of transposition of the element. In any case, if the distribution of IS5 is typical of other IS then it suggests that natural isolates of *E. coli* may carry up to 10 IS that are not represented in the genome of K-12. In this connection, it should be noted that 4 of 12 natural isolates were found to lack IS1, and among strains carrying IS1, there was extensive variation in size and number of IS1-hybridizing restriction fragments (12).

The variation in number of copies of IS5 and in size of IS5-bearing restriction fragments is extraordinary. Strains that have indistinguishable electrophoretic phenotypes of 11 diagnostic enzymes can usually be distinguished by their pattern of IS5 restriction fragments. Such variation usually reflects elements present at different sites in the genome (10, 11), but we cannot exclude some part of the variation being due to restriction site polymorphisms (14) or to elements located on plasmids that are present in some strains but not in

Table 3. Divergence between strains in prolonged storage

	Strain		
	M389 vs. M617	M609 vs. M611A	M679 vs. M680
Fragments			
Total	1 2	7 7	4 3
Common	1	5	3
Different	1	4	1
Year	1939	<1941	1934
Rate	0.0114	<0.0095	0.0034

Year represents year isolated; rate represents apparent transposition rate (rate/element/yr).

others. Nevertheless, because of this extensive variation, population studies of transposable elements should include as many independent isolates as feasible. The principal implication of variation in the number and genomic location of IS sequences is that such elements provide convenient genetic markers for detecting recent common ancestry among strains.

The occurrence of IS*5* in natural isolates of *E. coli* is associated with electrophoretic phenotype in that group I contains a greater proportion of strains carrying IS*5* than do groups II and III. Moreover, among strains carrying IS*5*, the average number of elements per strain is greater in group I than in groups II and III (though not statistically significant). The original separation into three groups was based on factor analysis of electrophoretic data (24). The groups are therefore defined on the basis of statistical similarity of electrophoretic phenotypes. However, the correlations with IS*5* provide independent support for the existence of underlying clusters of strains among natural isolates.

The Murray strains provide a unique opportunity to study the accumulation of transposable elements in cultures undergoing prolonged storage. The average number of IS*5* elements among Murray strains is 5.7 ± 1.2, which is significantly greater than the average of 3.2 ± 0.8 found among the ECOR group I strains, the subgroup of recent natural isolates with which the Murray strains are most similar. Three pairs of duplicates in the Murray collection provide a quantitative estimate of the apparent rate of transposition of IS*5* during prolonged storage, the average apparent rate being 0.008 ± 0.002 per IS*5* element per yr. This estimate pertains only to the standard storage procedures used by Murray, and it is an average. Transpositions may occur inhomogeneously in time, perhaps preferentially in the first years after storage. The rate of transposition may also differ according to the conditions of storage or in strains maintained by serial transfer. Since the effective generation time of strains undergoing storage in stab tubes is unknown, it is not possible to convert the transposition rate from years to generations. In addition, the estimated rate applies only to IS*5*, although comparisons of K-12 strains suggest that the average apparent rate of transposition of IS*1*, IS*2*, and IS*3* may be of the same order of magnitude (10). In any case, the estimated rate of transposition of IS*5* is sufficiently rapid to account for the differences observed among electrophoretically identical strains but sufficiently slow to maintain a close similarity among strains for at least 50 yr of storage.

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1. Calos, M. P. & Miller, J. H. (1980) *Cell* **20,** 579–595.
2. Kleckner, N. (1981) *Annu. Rev. Genet.* **15,** 341–404.
3. Campbell, A. (1981) *Annu. Rev. Microbiol.* **35,** 55–83.
4. Hartl, D. L., Dykhuizen, D. E. & Berg, D. E. (1984) in *Origins and Development of Adaptation*: Ciba Symposium 102, eds. Evered, D. & Collins, G. M. (Pitman, Bath, England), pp. 233–243.
5. Saedler, H., Cornelis, G., Cullum, J., Schumacher, B. & Sommer, H. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45,** 93–98.
6. Reynolds, A. E., Felton, J. & Wright, A. (1981) *Nature (London)* **293,** 625–629.
7. Ciampi, M. S., Schmid, M. B. & Roth, J. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5016–5020.
8. Simons, R. W., Hoopes, B. C., McClure, W. R. & Kleckner, N. (1983) *Cell* **34,** 673–682.
9. Hartl, D. L., Dykhuizen, D. E., Miller, R. D., Green, L. & de Framond, J. (1983) *Cell* **35,** 503–510.
10. Hu, M. & Deonier, R. C. (1981) *Gene* **16,** 161–170.
11. Schoner, B. & Schoner, R. G. (1981) *Gene* **16,** 347–352.
12. Nyman, K., Ohtsubo, H., Davison, D. & Ohtsubo, E. (1983) *Mol. Gen. Genet.* **189,** 516–518.
13. Anilionis, A. & Riley, M. (1980) *J. Bacteriol.* **143,** 355–365.
14. Harshman, L. & Riley, M. (1980) *J. Bacteriol.* **144,** 560–568.
15. Ochman, H. & Selander, R. K. (1984) *J. Bacteriol.* **157,** 690–693.
16. Hughes, V. M. & Datta, N. (1983) *Nature (London)* **302,** 725–726.
17. Engler, J. A. & van Bree, M. P. (1981) *Gene* **14,** 155–163.
18. Schoner, B. & Kahn, M. (1981) *Gene* **14,** 165–174.
19. Kroger, M. & Hobom, G. (1982) *Nature (London)* **297,** 159–162.
20. Rak, B., Lusky, M. & Hable, M. (1982) *Nature (London)* **297,** 124–128.
21. Schoner, B. (1983) *Gene* **21,** 203–210.
22. Jordan, B. A., Wolf, R. E., Jr., & Nasoff, M. (1982) *Plasmid* **8,** 98 (abstr.).
23. Nasoff, M. S. & Wolf, R. E., Jr. (1980) *J. Bacteriol.* **143,** 731–741.
24. Whittam, T. S., Ochman, H. & Selander, R. K. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1751–1755.
25. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.