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Clustered Third-Base Substitutions Among Wild Strains of *Escherichia coli*

Abstract. *Nucleotide sequences of translated regions of the* trp *operon in 12 wild strains of* Escherichia coli *reveal striking uniformity among eight strains (suggesting recent common ancestry and supporting the importance of periodic selection in natural populations) and clustered substitutions in four strains (implicating events affecting runs of nucleotides).*

Nucleotide sequences of homologous regions in different genera of bacteria (or in any long-separated genomes) may be expected to reveal adaptive differences, adaptive uniformity, and neutral variation. The neutral variation may represent both single and successive events at a single nucleotide site, given sufficient time of divergence from a common ancestor. In contrast, neutral differences observed between DNA sequences of independent isolates of a given species are likely in general to represent single events only. With this distinction in mind, we present some recent observations.

Nucleotide sequencing of the coding regions of the entire *trp* operon in *Escherichia coli* and *Salmonella typhimurium* has revealed third-base differences in 40 to 50 percent of all codons, but far fewer first- and second-base differences (*1*). These third-base differences appear to be distributed randomly, with the exception of one 21-codon stretch (as discussed below).

We have obtained from each of 12 wild *E. coli* strains a fragment of DNA extending at least from nucleotide 4343 in *trpC* through nucleotide 5990 in *trpA* (*2*). This 1648-bp segment, which includes all of *trpB*, corresponds to a deletion in the *trpCBA* region borne on a plasmid and filled by transduction (*3*). These fragments have been sequenced in large part and compared with *E. coli* strain K12. In contrast with the intergeneric (*Escherichia-Salmonella*) differences just mentioned, the nucleotide differences among the wild *E. coli* strains are highly clustered (Tables 1 and 2). Eight strains are remarkably similar: each differs from strain K12 by no more than one base in a thousand. Three other strains, identical in sequence so far, differ from K12 at ten sites. A final strain, 202I, differs from K12 at 44 sites. Thus the differences are not randomly distributed among these strains. Some base substitutions are clustered spatially also. For example, strain 45E has a set of four substitutions (4963 to 4975) in five consecutive codons in *trpB*. Strain 202I has the same set, as well as three more in a four-codon stretch 24 bases further along in *trpB*. In addition to these clusters there are other scattered substitutions, some shared between 45E and 202I and some not. Of the ten nucleotides in 45E that differ from K12, seven are shared by 202I. At the

Table 1. Characteristics of the wild *E. coli* strains tested. Bases were sequenced from positions 4321 through 5989.

Item	K12-like								45E-like			202I
Strain*	39A	66B	191F	200L	200M	201C	210J	217T	45E	70B	224H	202I
Source†	H, I	H, I	L, Z	P_1, N_1	P_1, N_1	P_2, N_1	A, Z	G, N_2	A, Z	R, Z	F, Z	A, Z
Number of bases sequenced	1300	1300	1200	1300	1400	1300	1100	1300	1300	950	1400	1300
Base change versus *E. coli* K12‡	4779 C → T	None	None	5922 C → A	None	5266 C → T	5114 T → G	5719 G → A	(10)	(10)	(10)	(44)
Electromorph§												
ADH	F	F	+	+	F	F	F	+	F	+	+	0
MDH	+	+	+	+	+	+	+	+	+	+	+	+
G6PD	+	+	+	+	+	+	F	+	+	+	+	+
6PGD	R	S	F	+	F	+	C	S	T	+	+	+
Thermostability class, 6PGD¶	S_4	S_3	S_3	+	S_4	+	S_2	+	+	N.T.	N.T.	N.T.

*Strain numbers follow (*2*). †Source symbols: H, human; L, leopard; P_1, domestic pig 1; P_2, domestic pig 2; A, Celebes black ape; G, domestic goat; R, lowland gorilla; F, giraffe; I, Iowa (State Hygienic Laboratory); N_1, first Indonesian sample; Z, Woodland Park Zoo, Seattle; N_2, second Indonesian sample. ‡Position and type. §ADH, alcohol dehydrogenase; MDH, malate dehydrogenase G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6 phosphogluconate dehydrogenase; mobilities [see (*2*)] are 0, no activity; C, R, S, T, +, and F are listed in the order of increasing mobility. ¶Thermostability categories: +, most common class; S, temperature-sensitive class, with subscript number rising with increasing sensitivity. Class differences are roughly 1 kcal/mole in activation energy of catalytic inactivation (*7*). Strains marked N.T. were not tested, but 95 percent of all 6PGDH "+" electromorphs tested were in the "+" thermostability class (*7*).

other three sites, 202I resembles K12, not 45E. Construction of a simple evolutionary tree suggests that the 4963 to 4975 sequence in K12 is not an ancestral one.

The mechanisms possibly responsible for the spatial clustering may be classified as follows. To begin with, some selective mechanism is clearly implied by the absence of first and second base substitutions in the clusters. Selection may further affect substitution in certain places because of some local noncoding function. Regions may vary considerably in their susceptibility to mutation. Finally, each cluster may in fact originate in an event (such as repair or recombination) affecting a run of nucleotides.

The 21-codon exception in the above comparison of *E. coli* K12 and *S. typhimurium* involves a region in *trpB* devoid of differences (*1*). Since 19 of the 21 codons involved have wobble alternatives, this identity is highly improbable if only individual random substitutions are responsible for all differences. Six wild *E. coli* strains have been sequenced in this exceptional region, and they are like K12 and *S. typhimurium*. *Shigella dysenteriae* [now considered a form of *E. coli* (*4*)] differs in two of the 19 third-position bases. This raises the possibility of a lateral transfer from *E. coli* to an ancestor of the tested strain of *S. typhimurium*, perhaps quite recently.

Our study was undertaken to explore the basic structural dynamics of the *E. coli* species genome in a theater believed to be essentially exempt from direct selection, namely third bases in translated regions for which no additional noncoding function is known. The high frequency of synonymous differences at third positions between *E. coli* and *S. typhimurium* is evidence of the neutrality of such alternatives. Moreover, the reciprocity of synonymous substitutions—such as TTT as against TTC in one place, and TTC for TTT (T, thymine; C, cytosine) elsewhere in the same cistron—is found in the *trpB* codons for almost every amino acid, and these reciprocal substitutions are not limited to wobble alternatives.

The findings of Selander and Levin and Whittam *et al.* (*5*) imply that selection plays a dominating indirect role in the distribution of genetic variation in *E. coli*. Hitchhiking, or periodic selection (*6*), in which a favorable mutation brings an entire genome to prominence before recombination can break the association, is implicated by the repeated appearance of certain complex electrophoretic genotypes in independently obtained *E. coli* strains (*5*). It thus appears that certain *E. coli* strains have spread rapidly (on an evolutionary time scale) throughout the world, resulting in the high likelihood that two strains picked at random have had a recent common ancestor.

The near-identity of eight sequenced strains (Table 1) supports this conclusion. It is of additional interest that no two of the eight are electrophoretically identical with respect to four enzymes tested previously (*5, 7*). The sequence identity between 45E, 70B, and 224H may be the trivial result of their common source (Woodland Park Zoo), but the K12 group affords no such explanation. As to the 45E and 202I sequences, the clustering of their differences argues against the estimation of divergence times from the proportion of substitutions (*8*), at least until more strains and perhaps more regions of the genome have been studied. Finally, it is not clear whether the clustering of differences among strains stems from the high communicability of *E. coli* and other enteric bacteria; it will be of interest to study bacteria with other life styles, such as the soil bacterium *Bacillus subtilis* (*9*).

Table 2. Base substitutions compared to K12 in strains 45E and 202I. Sites numbered as in Table 1. All substitutions are synonymous and at the third position unless marked. The phase changes between *trpC*, which ends at 4676, and *trpB*, which begins at 4688.

45E		202I
	(start 4343)	
	4346	T → C
	4349	G → T
	4358	A → G
T → C	4373	T → C
	4376	T → C
	4388	A → G
	4391	A → T
	4397	C → T
	4409	C → T
	4412	G → T
	4418	C → G
	4424	G → T*
*†T → C	4425	
	4448	T → A
	4451	A → C*
	4460	C → T
	4466	T → C
	4469	A → G
	4475	G → A
T → C	4484	
	4586	A → G
	4622	C → T
	4789	T → C
G → T	4903	
	4918	T → C
G → C	4963	G → C
G → T	4966	G → T
G → T	4972	G → T
G → A	4975	G → A
	4999	C → T
	5005	C → T
	5008	C → T
	(end 5046)	
	(start 5266)	
	5278	T → C
	5323	C → G
	5386	C → T
	(end 5392)	
	(start 5474)	
†C → T	5498	C → T†
T → C	5518	T → C
	5575	A → G
	5587	C → T
	5590	C → T
	5596	A → G
	5635	T → C
	5674	T → C
	5695	C → T
	5704	G → T
	5713	C → T
	5719	G → C
	(end 5767)	

*Amino acid substitution. †First-base substitution.

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8. If all differences were due to random neutral individual substitutions, the following simple kinetics would apply:

$$S \underset{k_2}{\overset{k_1}{\rightleftarrows}} D$$

where S is the proportion of nucleotides identical to the reference nucleotide and D is the proportion of differences; k_1 is mutation rate (of the order of 10^{-9} per specific substitution per generation), and k_2 is the sampling effect (negli-

gible here) plus the rate of loss of variation due to hitchhiking. Evolutionary divergence could be estimated under the same conditions as $t = [1/(k_1 + k_2)] \times \ln D_e/(D_e - D)$, where t is the number of generations since the last common ancestor and D_e is the proportion of differences expected at equilibrium. It is still quite possible that an objectively ascertainable subset of substitutions may be a proper subject for this sort of analysis.

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Forms of Memory Failure

Abstract. *Memory may fail in a variety of ways. Patients with Korsakoff's syndrome demonstrate global memory deficits similar to those seen in patients with early progressive dementia. Korsakoff's patients, however, may recall rules and principles for organizing information and can gain access to their previously acquired knowledge (semantic memory), whereas recent memory may be grossly impaired. In contrast, dementia patients may have little access to previously acquired knowledge and therefore have great difficulty in organizing and encoding ongoing events. These contrasting forms of memory failure have implications for understanding the structure and mechanisms of memory and learning, particularly the relationship between episodic and semantic memory, as well as the development of therapeutic strategies for cognitive impairments.*

Psychobiological determinants of memory failures remain poorly understood and are complex, variable in their form, and rarely complete. A variety of theoretical explanations have been proposed to account for memory failures in amnestic syndromes including disruptions in consolidation of memories (*1, 2*), acquisition and encoding processes (*3, 4*), retrieval processes (*1–5*), or unlinking some event from its processing context (dissociation of memory and awareness) (*6–9*). These explanations of memory failures are all concerned with episodic memory—memory for an event that has occurred at a particular time and place, and in a unique context. In contrast, semantic memory—the representation of structured and organized information, or knowledge in memory, which serves as a basis for appreciating, interpreting, and encoding ongoing experience—may be a separate, distinct memory system (*10, 11*); access to that type of memory is a necessary condition for forming new memories. This study was designed to contrast and relate aspects of episodic and semantic memory by comparing the determinants of comparably memory-impaired patients with Korsakoff's disease (KD) and those in an early stage of a progressive degenerative dementia (PD), probably of an Alzheimer's type (a diagnosis confirmable only on the basis of neuropathological findings).

The most common amnestic syndrome is KD; although these patients have been frequently studied because of the presumed "purity" of their amnesia, they, nevertheless, can learn and remember some information (*4*). For example, although they are often incapable of remembering previously processed events on demand (declarative memory), they, like some other amnestic patients, are capable of learning and then "remembering" (performing) many complex tasks (*5, 12–15*). In addition they are able to remember, on demand, at least some recently occurring events and are also sensitive to conditions that would ordinarily affect recall in unimpaired subjects (*16*). Patients with PD can demonstrate functionally equivalent memory failures. The impairment of PD patients, unlike that of KD patients, is progressive, owing to continued neuropathological deterioration, and also seems related to how well these patients can obtain access to and use their previously acquired knowledge in processing ongoing events. We hypothesized that KD patients with severe impairments in recent memory could nonetheless effectively gain access to knowledge structures in semantic memory. In contrast we predicted that semantic memory functions would be impaired in PD patients and the extent of that impairment would be directly related to the severity of episodic memory failures. That is, unlike KD patients, PD patients were predicted to fail to remember because they cannot gain access to knowledge structures necessary for appreciating and encoding ongoing events.

The study was completed in two stages. Patients selected for further study were first evaluated neurologically, psychiatrically, and neuropsychologically. From this sample, patients were chosen in matched pairs on the basis of demographic characteristics, as well as comparable impairments in memory. Memory impairments were measured both clinically and psychometrically, as well as with laboratory tests of learning and memory. The psychometric evaluation and measures used to match the memory impairment of KD and PD patients included the Wechsler Memory Scale (WMS) performance, a standard global clinical assessment procedure that relates memory performance to measures of intelligence (IQ). The premorbid IQ of PD patients was estimated to be well above average on the basis of weighted factors including age, sex, education, and occupation (*17*) and was not

Table 1. Characteristics of patients with progressive degenerative dementia (PD) and Korsakoff's disease (KD), presented as means ± standard errors.

Characteristic	Group		
	PD (N = 8)	KD (N = 8)	Control (N = 8)
	Demographic measures		
Age (years)	58 ± 3.2	57 ± 6.1	61 ± 4.4
Education (years)	15.7 ± 2.7	11.0 ± 2.5	13.6 ± 2.2
	Psychometric measures		
Wechsler memory scale	72.4 ± 8.1	73.3 ± 10.6	103.6 ± 7.2
	Experimental measures of episodic memory		
Prompted recall			
Recall of words (No.)			
Trials 1 to 5	4.0 ± 0.2	4.2 ± 0.4	7.3 ± 0.6
Trials 6 to 10	4.6 ± 0.3	4.9 ± 0.4	9.7 ± 0.8
Consistency of recall (proportion)			
Trials 1 to 5	0.19 ± 0.03	0.22 ± 0.05	0.72 ± 0.08
Trials 6 to 10	0.24 ± 0.04	0.27 ± 0.06	0.84 ± 0.07
Proportion recalled (of 20)			
Words	0.07 ± 0.04	0.08 ± 0.03	0.42 ± 0.08
Pictures	0.15 ± 0.05	0.11 ± 0.04	0.48 ± 0.01
	Frequency monitoring		
Index*	0.24 ± 0.008	0.26 ± 0.008	1.5 ± 0.01

*Discrimination between frequently and infrequently presented words. A score of 0 indicates inability to accomplish frequency judgments in memory.