Table S1. Fitting polynomial equations to ACE$_u$ distributions.

	E. coli to *S. enterica*		*S. enterica* to *E. coli*	
Polynomial Order	Pearson R	ΔR[a]	Pearson R	ΔR
1	0.8885		0.8885	
2	0.9028	0.0143	0.8892	0.0007
3	0.9029	0.0001	0.8942	0.0050
4	0.9029	0.0001	0.8952	0.0010
5	0.9029	0.0000	0.8957	0.0005
6	0.9030	0.0001	0.8960	0.0003

a. Difference between successive R values.

Table S2. Pearson R values for second-order polynomial regression of ACE values of 2235 orthologous genes among 8 taxa. The independent variable is listed on the left, while the dependent variable is listed across the top. The upper table reports correlation of ACE_z values, while the lower table from the correlations for $ACE_{\underline{u}}$ values.

ACE_z	Eco[a]	Efe	Sty	Saz	Cro	Cko	Ecl	Ctu
Eco	1.000	0.958	0.910	0.904	0.885	0.913	0.893	0.841
Efe	0.959	1.000	0.905	0.898	0.869	0.904	0.879	0.822
Sty	0.905	0.898	1.000	0.970	0.902	0.919	0.883	0.851
Saz	0.898	0.890	0.970	1.000	0.896	0.918	0.877	0.853
Cro	0.879	0.859	0.900	0.894	1.000	0.905	0.889	0.887
Cko	0.913	0.901	0.923	0.922	0.911	1.000	0.913	0.872
Ecl	0.896	0.879	0.891	0.887	0.899	0.916	1.000	0.896
Ctu	0.830	0.807	0.843	0.845	0.882	0.858	0.881	1.000

ACE_u	Eco	Efe	Sty	Saz	Cro	Cko	Ecl	Ctu
Eco	1.000	0.950	0.903	0.894	0.885	0.906	0.884	0.852
Efe	0.953	1.000	0.900	0.891	0.876	0.898	0.871	0.841
Sty	0.889	0.880	1.000	0.967	0.904	0.907	0.873	0.865
Saz	0.879	0.869	0.966	1.000	0.897	0.905	0.866	0.862
Cro	0.861	0.843	0.892	0.886	1.000	0.892	0.878	0.887
Cko	0.898	0.842	0.912	0.910	0.909	1.000	0.899	0.878
Ecl	0.881	0.862	0.884	0.879	0.901	0.905	1.000	0.895
Ctu	0.818	0.795	0.844	0.842	0.882	0.851	0.867	1.000

a. Taxa are: Eco, *Escherichia coli;* Efe, *E. fergusonii;* Sty, *Salmonella enterica* Typhimurium; Saz, *S. enterica* Arizonae; Cro, *Citrobacter rodentium*; Cko, *C. koseri*; Ecl, *Enterobacter cloacae; Ctu, Cronobacter turicensis.*

Table S3. ANOVA variances for differences in ACE$_u$ values for 1166 genes within and between 390 operons among 17 genomes.

	Cko	Cro	Ctu	Dze	Ecl	Esp	Eam	Eta	Eco	Efe	Kpn	Kva	Pwa	Saz	Sty	Spr	Yen
Cko	3.64E-50	3.10E-02	1.59E-05	9.98E-07	3.91E-05	9.38E-05	2.19E-06	3.62E-06	1.79E-02	1.23E-05	1.92E-04	2.69E-06	1.64E-04	3.39E-01	6.09E-02	2.02E-03	4.08E-06
Cro		3.79E-62	1.83E-02	4.23E-07	3.85E-06	5.09E-07	3.91E-03	2.07E-03	3.32E-06	4.95E-10	9.23E-03	1.05E-02	9.39E-07	6.59E-03	6.60E-05	1.06E-03	2.58E-05
Ctu			4.82E-72	6.18E-11	7.54E-06	4.33E-06	2.09E-02	6.88E-03	4.78E-10	3.02E-13	8.68E-03	5.55E-03	4.82E-08	2.37E-04	1.67E-06	6.06E-04	2.12E-06
Dze				1.41E-53	1.42E-07	1.72E-08	7.76E-05	4.37E-05	2.20E-11	7.00E-14	2.79E-08	1.41E-08	5.21E-04	1.29E-04	4.02E-05	1.94E-07	3.56E-11
Ecl					2.52E-46	1.97E-03	6.08E-05	6.60E-05	1.63E-03	5.76E-07	1.97E-04	3.31E-04	5.30E-05	1.75E-03	4.48E-04	3.49E-03	4.87E-03
Esp						1.16E-47	5.62E-04	1.46E-05	5.91E-05	3.98E-08	2.65E-05	5.02E-05	7.54E-06	1.16E-02	9.43E-04	1.78E-05	2.02E-04
Eam							1.67E-47	3.39E-01	1.29E-12	5.45E-14	8.50E-03	1.56E-03	1.52E-03	2.16E-08	3.61E-08	4.49E-02	1.48E-04
Eta								6.95E-56	1.90E-13	1.19E-15	6.45E-03	1.88E-03	9.13E-05	3.41E-07	2.83E-07	1.04E-01	8.47E-06
Eco									1.38E-39	2.26E-02	1.83E-08	4.32E-10	8.29E-08	7.03E-03	7.18E-03	1.07E-07	4.36E-03
Efe										9.10E-41	4.49E-09	5.08E-12	2.88E-10	4.08E-03	2.98E-03	5.48E-10	9.90E-04
Kpn											5.56E-70	8.11E-01	2.72E-05	4.90E-02	2.60E-03	4.34E-02	2.98E-04
Kva												3.28E-77	6.51E-07	2.56E-04	1.72E-06	1.42E-02	1.13E-06
Pwa													7.03E-47	1.33E-04	1.16E-05	1.46E-05	6.33E-06
Saz														1.49E-59	1.97E-01	3.74E-03	3.06E-04
Sty															9.19E-63	5.35E-04	3.06E-04
Spr																4.86E-48	5.91E-04
Yen																	8.46E-38

a. Taxa are: Cko, *Citrobacter koseri;* Cro, *Citrobacter rodentium; Ctu, Cronobacter turicensis*; Dze, *Dickeya zeae*; Ecl, *Enterobacter cloacae*; Esp, *Enterobacter* sp. 638; Eam, *Er. amylovora*; Eta, *Erwinia tasmaniensis*; Eco, *Escherichia coli;* Efe, *E. fergusonii;* Kpn, *K. pneumonia*; Kva, *Klebsiella varicola*; Pwa, *Pectobacterium wasabiae*; Sty, *Salmonella enterica* Typhimurium; Saz, *S. enterica* Arizonae; Spr, *Serratia proteamaculans;* Yen, *Yersinia enterocolitica*.

b. Values on the diagonal report P-values of the ANOVA F statistic testing for similarity among ACE$_u$ values for genes in the same operon. Significant values indicate clustering of genes with similar ACE$_u$ values.

c. Values off the diagonal report P-values of the ANOVA F statistic testing for differences in the ACE$_u$ values for cognate operons in different species. Significant values indicate a change in the ACE$_u$ value between species.

Table S4. Operons with significant change in ACE$_u$ values between species. A total of 523 operons were examined.

	$P < 0.05$			$P < 0.01$			$P < 0.002$		
Comparison	Count	Fraction	Excess	Count	Fraction	Excess	Count	Fraction	Excess
Cro-Cko[a]	29[b]	0.055[c]	1.11[d]	6	0.011	1.15	4	0.008	3.82
Cro-Ecl	46	0.088	1.76	15	0.029	2.87	7	0.013	6.69
Cro-Eco	81	0.155	3.10	39	0.075	7.46	21	0.040	20.08
Cro-Sen	53	0.101	2.03	19	0.036	3.63	10	0.019	9.56
Cro-Sty	46	0.088	1.76	14	0.027	2.68	7	0.013	6.69
Cro-Efe	98	0.187	3.75	51	0.098	9.75	26	0.050	24.86
Cro-Ctu	67	0.128	2.56	31	0.059	5.93	18	0.034	17.21
Cko-Ecl	42	0.080	1.61	15	0.029	2.87	9	0.017	8.60
Cko-Eco	59	0.113	2.26	27	0.052	5.16	13	0.025	12.43
Cko-Sen	24	0.046	0.92	4	0.008	0.76	1	0.002	0.96
Cko-Sty	18	0.034	0.69	5	0.010	0.96	3	0.006	2.87
Cko-Efe	65	0.124	2.49	26	0.050	4.97	15	0.029	14.34
Cko-Ctu	89	0.170	3.40	48	0.092	9.18	30	0.057	28.68
Ecl-Eco	81	0.155	3.10	35	0.067	6.69	21	0.040	20.08
Ecl-Sen	66	0.126	2.52	35	0.067	6.69	21	0.040	20.08
Ecl-Sty	69	0.132	2.64	32	0.061	6.12	16	0.031	15.30
Ecl-Efe	92	0.176	3.52	42	0.080	8.03	24	0.046	22.94
Ecl-Ctu	56	0.107	2.14	24	0.046	4.59	15	0.029	14.34
Eco-Sen	60	0.115	2.29	21	0.040	4.02	7	0.013	6.69
Eco-Sty	61	0.117	2.33	20	0.038	3.82	10	0.019	9.56
Eco-Efe	9	0.017	0.34	2	0.004	0.38	1	0.002	0.96
Eco-Ctu	122	0.233	4.67	78	0.149	14.91	55	0.105	52.58
Sen-Sty	2	0.004	0.08	1	0.002	0.19	0	0.000	0.00
Sen-Efe	61	0.117	2.33	26	0.050	4.97	10	0.019	9.56
Sen-Ctu	110	0.210	4.21	67	0.128	12.81	43	0.082	41.11
Sty-Efe	62	0.119	2.37	29	0.055	5.54	11	0.021	10.52
Sty-Ctu	104	0.199	3.98	65	0.124	12.43	44	0.084	42.07
Efe-Ctu	125	0.239	4.78	78	0.149	14.91	62	0.119	59.27

a. Taxon designations as in Table S3.

b. The number of operons whose ACE$_u$ value exceeds the critical probability.

c. The fraction of the genome represented by the operons with excess difference in ACE$_u$ values.

d. The ratio of the number of operons with the given level of ACE$_u$ difference to the number expected.

Table S5. Operons and genes with significant change in ACE$_u$ values between *E. coli* and *S. enterica*. A total of 665 operons and 3072 individual genes were examined.

	Number of Operons				
Threshold	Observed	Expected	Excess	Fraction Observed	Fold Excess
0.2	200	133.0	67.0	0.301	1.50
0.1	126	66.5	59.5	0.189	1.89
0.05	82	33.3	48.8	0.123	2.47
0.02	51	13.3	37.7	0.077	3.83
0.01	33	6.7	26.4	0.050	4.96
0.005	21	3.3	17.7	0.032	6.32
0.002	15	1.3	13.7	0.023	11.28
0.001	15	0.7	14.3	0.023	22.56

	Number of Genes				
Threshold	Observed	Expected	Excess	Fraction Observed	Fold Excess
0.2	170	133.0	37.0	0.256	1.28
0.1	106	66.5	39.5	0.159	1.59
0.05	63	33.3	29.8	0.095	1.89
0.02	40	13.3	26.7	0.060	3.01
0.01	32	6.7	25.4	0.048	4.81
0.005	20	3.3	16.7	0.030	6.02
0.002	11	1.3	9.7	0.017	8.27
0.001	10	0.7	9.3	0.015	15.04

Table S6. Tetrad Analyses. Relationships were inferred from sets of orthologous genes found in species residing in different habitats.

Species	Family	Environment	Tetrad Supported	
			Proteins[a]	Codon Usage[b]
Bifidobacterium longum	Bifidobacteriaceae	Gastrointestinal	(Cin,Cge), (Blo,Mcu)	(Blo,Cin), (Mcu,Cge)
Collinsella intestinalis	Coriobacteriaceae	Gastrointestinal		
Mobiluncus curtisii	Actinomycetaceae	Urogenital		
Corynebacterium genitalium	Corynebacteriaceae	Urogenital		
Staphylococcus aureus	Staphylococcaceae	Skin	(Sau,Edo), (Pan,Chy)	(Sau,Pan), (Chy,Edo)
Peptostreptococcus anaerobius	Peptostreptococcaceae	Skin		
Clostridium hylemonae	Clostridiaceae	Gastrointestinal		
Eubacterium dolichum	Eubacteriaceae	Gastrointestinal		
Oribacterium sinus	Lachnospiraceae	Oral	(Osi,Lre), (Gel,Efa)	(Osi,Gel), (Lre,Efa)
Granulicatella elegans	Carnobacteriaceae	Oral		
Lactobacillus reuteri	Lactobacillaceae	Gastrointestinal		
Enterococcus faecalis	Enterococcaceae	Gastrointestinal		

a. Neighbor-Joining tree were inferred from overall similarity of orthologous proteins.
b. Neighbor-Joining trees were inferred from correlations of ACE_u values among orthologous protein-coding genes.

Table S7. Excess of genes showing changes in codon selection as a function of strand identity among 2235 genes shared among 8 taxa.

Comparison	Total Genes		Genes with significant change in ACE_u ($P < 0.01$)		Fraction of Genes With Significant Change in ACE_u	
	Same Strand	Different Strand	Same Strand	Different Strand	All Genes	Same Strand Genes
Cro-Cko[a]	2068	167	32	2	0.015	0.015
Cro-Ecl	2064	171	27	5	0.014	0.013
Cro-Eco	2068	167	111	11	0.055	0.054
Cro-Sen	2066	169	37	3	0.018	0.018
Cro-Sty	2066	169	32	6	0.017	0.015
Cro-Efe	2072	163	108	10	0.053	0.052
Cro-Ctu	2065	170	54	8	0.028	0.026
Cko-Ecl	2229	6	43	0	0.019	0.019
Cko-Eco	2233	2	68	0	0.030	0.030
Cko-Sen	2231	4	20	1	0.009	0.009
Cko-Sty	2231	4	23	1	0.011	0.010
Cko-Efe	2229	6	71	0	0.032	0.032
Cko-Ctu	2220	15	103	1	0.047	0.046
Ecl-Eco	2227	8	111	1	0.050	0.050
Ecl-Sen	2225	10	72	1	0.033	0.032
Ecl-Sty	2225	10	75	1	0.034	0.034
Ecl-Efe	2223	12	125	0	0.056	0.056
Ecl-Ctu	2216	19	64	0	0.029	0.029
Eco-Sen	2231	4	64	2	0.030	0.029
Eco-Sty	2231	4	69	2	0.032	0.031
Eco-Efe	2229	6	5	1	0.003	0.002
Eco-Ctu	2220	15	205	1	0.092	0.092
Sen-Sty	2233	2	2	0	0.001	0.001
Sen-Efe	2227	8	69	2	0.032	0.031
Sen-Ctu	2218	17	111	2	0.051	0.050
Sty-Efe	2229	6	60	2	0.028	0.027
Sty-Ctu	2218	17	131	3	0.060	0.059
Efe-Ctu	2216	19	228	2	0.103	0.103

a. Taxon designations as in Table S3.

Table S8. Excess of genes showing changes in codon selection as a function of support for species tree among 2235 genes shared among 8 taxa.

	Genes with significant change in ACE_u ($P < 0.01$)			Fraction of Genes With Significant Change in ACE_u	
Comparison	All Genes	Native Genes	Potentially Xenologous Genes[b]	All Genes	Native genes
Cro-Cko[a]	34	33	1	0.015	0.015
Cro-Ecl	32	32	0	0.014	0.014
Cro-Eco	122	121	1	0.055	0.055
Cro-Sen	40	40	0	0.018	0.018
Cro-Sty	38	38	0	0.017	0.017
Cro-Efe	118	115	3	0.053	0.052
Cro-Ctu	62	62	0	0.028	0.028
Cko-Ecl	43	42	1	0.019	0.019
Cko-Eco	68	65	3	0.030	0.029
Cko-Sen	21	21	0	0.009	0.010
Cko-Sty	24	22	2	0.011	0.010
Cko-Efe	71	68	3	0.032	0.031
Cko-Ctu	104	103	1	0.047	0.047
Ecl-Eco	112	111	1	0.050	0.050
Ecl-Sen	73	72	1	0.033	0.033
Ecl-Sty	76	75	1	0.034	0.034
Ecl-Efe	125	123	2	0.056	0.056
Ecl-Ctu	64	64	0	0.029	0.029
Eco-Sen	66	64	2	0.030	0.029
Eco-Sty	71	69	2	0.032	0.031
Eco-Efe	6	4	2	0.003	0.002
Eco-Ctu	206	204	2	0.092	0.092
Sen-Sty	2	2	0	0.001	0.001
Sen-Efe	71	70	1	0.032	0.032
Sen-Ctu	113	111	2	0.051	0.050
Sty-Efe	62	61	1	0.028	0.028
Sty-Ctu	134	133	1	0.060	0.060
Efe-Ctu	230	227	3	0.103	0.103

a. Taxon designations as in Table S3.

b. The 25 genes rejecting the species tree at $P < 0.01$ are homologs of the *E. coli* genes *accB, acrF, argE, bamA, btuB*, dnaB, eptB, fabB, fadL*, fepA*, fhuA*, fliD, glmS, glnP, mazG, murA, purA, rfe, rpsN, rsd, suhB, tppB, ybaP, ycbB* and *yeeF*. Genes marked with an asterisk are outer-membrane proteins (Table S9).

Table S9. Excess of genes showing changes in codon selection as a function of protein product location among 2235 genes shared among 8 taxa.

	Genes with significant change in ACE_u ($P < 0.01$)			Fraction of Genes With Significant Change in ACE_u	
Comparison	All Genes	Non-OMP genes	OMP Genes[b]	All Genes	Non-OMP genes
Cro-Cko[a]	34	31	3	0.015	0.014
Cro-Ecl	32	30	2	0.014	0.014
Cro-Eco	122	118	4	0.055	0.053
Cro-Sen	40	40	0	0.018	0.018
Cro-Sty	38	38	0	0.017	0.017
Cro-Efe	118	114	4	0.053	0.052
Cro-Ctu	62	61	1	0.028	0.028
Cko-Ecl	43	41	2	0.019	0.019
Cko-Eco	68	64	4	0.030	0.029
Cko-Sen	21	21	0	0.009	0.010
Cko-Sty	24	23	1	0.011	0.010
Cko-Efe	71	68	3	0.032	0.031
Cko-Ctu	104	102	2	0.047	0.046
Ecl-Eco	112	107	5	0.050	0.048
Ecl-Sen	73	72	1	0.033	0.033
Ecl-Sty	76	74	2	0.034	0.034
Ecl-Efe	125	121	4	0.056	0.055
Ecl-Ctu	64	62	2	0.029	0.028
Eco-Sen	66	63	3	0.030	0.029
Eco-Sty	71	67	4	0.032	0.030
Eco-Efe	6	4	2	0.003	0.002
Eco-Ctu	206	202	4	0.092	0.092
Sen-Sty	2	2	0	0.001	0.001
Sen-Efe	71	70	1	0.032	0.032
Sen-Ctu	113	112	1	0.051	0.051
Sty-Efe	62	61	1	0.028	0.028
Sty-Ctu	134	133	1	0.060	0.060
Efe-Ctu	230	228	2	0.103	0.103

a. Taxon designations as in Table S3.

b. Homologs of the *E. coli* genes *bamC, blc, btuB*, fadL*, fepA*, fhuA*, hofQ, lamB, mlaA, mltA, mltB, mltC, mltD, nfrA, ompA, ompC, ompW, ompX, pal, pgpB, skp, slyB, tolC, tsx, wza, ybhC* and *yeaY*. The phylogeny of genes marked with an asterisk reject the species tree at P<0.01 (Table S8).