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Evolution

Genome signature comparisons among prokaryote, plasmid, and mitochondrial DNA

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ABSTRACT Our basic observation is that each genome has a characteristic "signature" defined as the ratios between the observed dinucleotide frequencies and the frequencies expected if neighbors were chosen at random (dinucleotide relative abundances). The remarkable fact is that the signature is relatively constant throughout the genome; i.e., the patterns and levels of dinucleotide relative abundances of every 50-kb segment of the genome are about the same. Comparison of the signatures of different genomes provides a measure of similarity which has the advantage that it looks at all the DNA of an organism and does not depend on the ability to align homologous sequences of specific genes. Genome signature comparisons show that plasmids, both specialized and broad-range, and their hosts have substantially compatible (similar) genome signatures. Mammalian mitochondrial (Mt) genomes are very similar, and animal and fungal Mt are generally moderately similar, but they diverge significantly from plant and protist Mt sets. Moreover, Mt genome signature differences between species parallel the corresponding nuclear genome signature differences, despite large differences between Mt and host nuclear signatures. In signature terms, we find that the archaea are not a coherent clade. For example, *Sulfolobus* and *Halobacterium* are extremely divergent. There is no consistent pattern of signature differences among thermophiles. More generally, grouping prokaryotes by environmental criteria (e.g., habitat propensities, osmolarity tolerance, chemical conditions) reveals no correlations in genome signature.

Extensive data support the proposal that each living organism possesses a genomic signature consisting of dinucleotide relative abundance values calculated from genomic sequences (1–3). Explicitly, the genomic signature profile consists of the array $\{\rho^*_{XY} = f^*_{XY}/f^*_X f^*_Y\}$, where f^*_X denotes the frequency of the mononucleotide X and f^*_{XY} the frequency of the dinucleotide XY, both computed from the sequence concatenated with its inverted complement. These dinucleotide relative abundance values $\{\rho^*_{XY}\}$ minus 1 (also termed dinucleotide biases) effectively assess differences between the observed dinucleotide frequencies and those expected from random associations of the component mononucleotide frequencies. From data simulations and statistical theory, the estimates $\rho^*_{XY} \leq 0.78$ or $\rho^*_{XY} \geq 1.23$ convey significant underrepresentation or overrepresentation, respectively, for 50-kb random DNA contigs (1–3). We present substantial data showing that the genome signatures of bacterial plasmids are pervasively similar to those of their natural hosts. By contrast, the signatures of animal mitochondrial DNA are not close to those of their hosts but are generally concordant with those of other animal mitochondrial (Mt) DNA.

Justifications for Using Genome Signature. Biochemical experiments in the 1960s and 1970s measuring nearest-neighbor frequencies (4, 5) established that the set of dinucleotide relative abundance values $\{\rho^*_{XY}\}$ is a remarkably stable property of the DNA of an organism. From this perspective, the set of dinucleotide relative abundance values constitutes a *genomic signature* that is diagnostic and can discriminate sequences from different organisms (3, 6). What causes the uniformity of signature throughout the genome? It pervades both noncoding and coding DNA (7), and hence cannot be explained by preferential codon usage. A reasonable explanation postulates differences in the replication and repair machinery of different species, which either preferentially generate or preferentially select specific dinucleotides in the DNA. These effects might operate through local DNA structures (base step conformational tendencies), context-dependent mutation rates, methylation, and/or other DNA modifications (1–3, 6).

A measure of genomic signature difference between two sequences f and g (from different organisms or from different regions of the same genome) is the average absolute dinucleotide relative abundance difference calculated as

$$\delta^*(f, g) = 1/16 \sum |\rho^*_{XY}(f) - \rho^*_{XY}(g)| \qquad (\delta^* \text{ difference}),$$

where the sum extends over all dinucleotides. Levels of δ^* differences of 50-kb contigs for some reference examples are described in the Introduction of the accompanying paper (8).

Genome Signature Comparisons. Fig. 1 displays the genome signature profiles for all currently available extensive genome sequence sets. These include 22 eubacterial genome ensembles (mostly complete genomes), 5 archaeal chromosomes, the *Saccharomyces cerevisiae* and *Caenorhabditis elegans* complete genomes, and extensive nonredundant sequence collections from 5 other eukaryotic species. For each genome, the dinucleotide biases $\{\rho^*_{XY}\}$ are substantially invariant (9).

The dinucleotide TA is broadly underrepresented or low normal in prokaryotic sequences about the level $0.50 \leq \rho^*_{TA} \leq 0.82$ (exceptions include *Rickettsia prowazekii, Clostridium acetobutylicum*, and the archaea *P. aerophilum, P. horikoshii*, and *Sulfolobus* sp. (full names appear in the legend to Figs. 1 and 2). TA underrepresentation is also pervasive in eukaryotic chromosomes (exception *P. falciparum*) but not in eukaryotic small viral genomes or in animal mitochondrial genomes (10, 11).

Among prokaryotes, CG is suppressed (underrepresented) in *M. genitalium* (but not in *M. pneumoniae*), in *R. prowazekii*, in *B. burgdorferi*, in *C. jejuni*, in the low-G+C Gram-positive sequences of *Streptococcus* and *Clostridium*, and in several thermophiles, including *M. jannaschii*, *M. thermoautotrophicum*, *Sulfolobus* spp., but not in *P. aerophilum* or *P. horikoshii.* At the other extreme, CG is overrepresented in *B. stearothermophilus*, in halobacteria, and also in several β- and α-proteobacterial genomes (e.g., *Neisseria* spp. and *Rhizobium* spp.). Among eukaryotes, CG shows potent suppression in vertebrates (even in deuterostomes). Overall, ρ^*_{CG} values in verte-

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Abbreviation: Mt, mitochondria(l).

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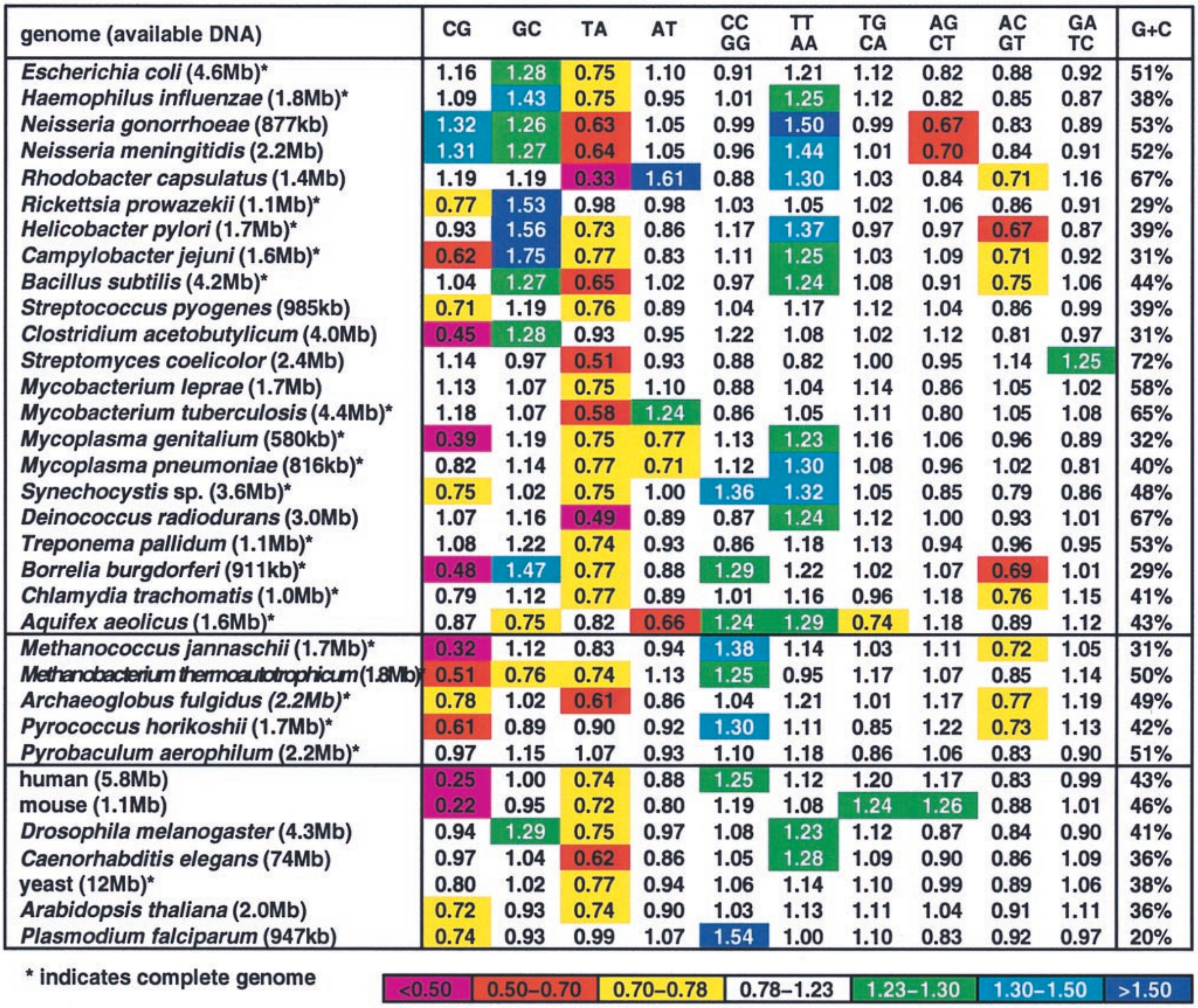


genome (available DNA)	CG	GC	TA	AT	CC GG	TT AA	TG CA	AG CT	AC GT	GA TC	G+C
Escherichia coli (4.6Mb)*	1.16	1.28	0.75	1.10	0.91	1.21	1.12	0.82	0.88	0.92	51%
Haemophilus influenzae (1.8Mb)*	1.09	1.43	0.75	0.95	1.01	1.25	1.12	0.82	0.85	0.87	38%
Neisseria gonorrhoeae (877kb)	1.32	1.26	0.63	1.05	0.99	1.50	0.99	0.67	0.83	0.89	53%
Neisseria meningitidis (2.2Mb)	1.31	1.27	0.64	1.05	0.96	1.44	1.01	0.70	0.84	0.91	52%
Rhodobacter capsulatus (1.4Mb)	1.19	1.19	0.33	1.61	0.88	1.30	1.03	0.84	0.71	1.16	67%
Rickettsia prowazekii (1.1Mb)*	0.77	1.53	0.98	0.98	1.03	1.05	1.02	1.06	0.86	0.91	29%
Helicobacter pylori (1.7Mb)*	0.93	1.56	0.73	0.86	1.17	1.37	0.97	0.97	0.67	0.87	39%
Campylobacter jejuni (1.6Mb)*	0.62	1.75	0.77	0.83	1.11	1.25	1.03	1.09	0.71	0.92	31%
Bacillus subtilis (4.2Mb)*	1.04	1.27	0.65	1.02	0.97	1.24	1.08	0.91	0.75	1.06	44%
Streptococcus pyogenes (985kb)	0.71	1.19	0.76	0.89	1.04	1.17	1.12	1.04	0.86	0.99	39%
Clostridium acetobutylicum (4.0Mb)	0.45	1.28	0.93	0.95	1.22	1.08	1.02	1.12	0.81	0.97	31%
Streptomyces coelicolor (2.4Mb)	1.14	0.97	0.51	0.93	0.88	0.82	1.00	0.95	1.14	1.25	72%
Mycobacterium leprae (1.7Mb)	1.13	1.07	0.75	1.10	0.88	1.04	1.14	0.86	1.05	1.02	58%
Mycobacterium tuberculosis (4.4Mb)*	1.18	1.07	0.58	1.24	0.86	1.05	1.11	0.80	1.05	1.08	65%
Mycoplasma genitalium (580kb)*	0.39	1.19	0.75	0.77	1.13	1.23	1.16	1.06	0.96	0.89	32%
Mycoplasma pneumoniae (816kb)*	0.82	1.14	0.77	0.71	1.12	1.30	1.08	0.96	1.02	0.81	40%
Synechocystis sp. (3.6Mb)*	0.75	1.02	0.75	1.00	1.36	1.32	1.05	0.85	0.79	0.86	48%
Deinococcus radiodurans (3.0Mb)	1.07	1.16	0.49	0.89	0.87	1.24	1.12	1.00	0.93	1.01	67%
Treponema pallidum (1.1Mb)*	1.08	1.22	0.74	0.93	0.86	1.18	1.13	0.94	0.96	0.95	53%
Borrelia burgdorferi (911kb)*	0.48	1.47	0.77	0.88	1.29	1.22	1.02	1.07	0.69	1.01	29%
Chlamydia trachomatis (1.0Mb)*	0.79	1.12	0.77	0.89	1.01	1.16	0.96	1.18	0.76	1.15	41%
Aquifex aeolicus (1.6Mb)*	0.87	0.75	0.82	0.66	1.24	1.29	0.74	1.18	0.89	1.12	43%
Methanococcus jannaschii (1.7Mb)*	0.32	1.12	0.83	0.94	1.38	1.14	1.03	1.11	0.72	1.05	31%
Methanobacterium thermoautotrophicum (1.8Mb)	0.51	0.76	0.74	1.13	1.25	0.95	1.17	1.07	0.85	1.14	50%
Archaeoglobus fulgidus (2.2Mb)*	0.78	1.02	0.61	0.86	1.04	1.21	1.01	1.17	0.77	1.19	49%
Pyrococcus horikoshii (1.7Mb)*	0.61	0.89	0.90	0.92	1.30	1.11	0.85	1.22	0.73	1.13	42%
Pyrobaculum aerophilum (2.2Mb)*	0.97	1.15	1.07	0.93	1.10	1.18	0.86	1.06	0.83	0.90	51%
human (5.8Mb)	0.25	1.00	0.74	0.88	1.25	1.12	1.20	1.17	0.83	0.99	43%
mouse (1.1Mb)	0.22	0.95	0.72	0.80	1.19	1.08	1.24	1.26	0.88	1.01	46%
Drosophila melanogaster (4.3Mb)	0.94	1.29	0.75	0.97	1.08	1.23	1.12	0.87	0.84	0.90	41%
Caenorhabditis elegans (74Mb)	0.97	1.04	0.62	0.86	1.05	1.28	1.09	0.90	0.86	1.09	36%
yeast (12Mb)*	0.80	1.02	0.77	0.94	1.06	1.14	1.10	0.99	0.89	1.06	38%
Arabidopsis thaliana (2.0Mb)	0.72	0.93	0.74	0.90	1.03	1.13	1.11	1.04	0.91	1.11	36%
Plasmodium falciparum (947kb)	0.74	0.93	0.99	1.07	1.54	1.00	1.10	0.83	0.92	0.97	20%

* indicates complete genome

<0.50	0.50–0.70	0.70–0.78	0.78–1.23	1.23–1.30	1.30–1.50	>1.50

FIG. 1. Genome signature (dinucleotide relative abundances) of complete genomes and large DNA sequence samples (>500 kb).

brates range from 0.23 to 0.40, whereas they are in the normal range for insects, worms, and most fungi. It has been shown (12) that unmethylated CG dinucleotides of normal frequency in most enteroproteobacteria can induce an immune response in mammalian genomes, where CG is very low. Is this a concomitant of genomic signature biases? The reverse dinucleotide, GC, is predominantly overrepresented in many β- and γ-proteobacterial sequences and in several low-G+C Gram-positive bacterial genomes (e.g., *B. subtilis* and *C. acetobutylicum*; Fig. 1). In eukaryotes, moderate overrepresentation of GC occurs in *Drosophila* species but apparently not in other metazoa.

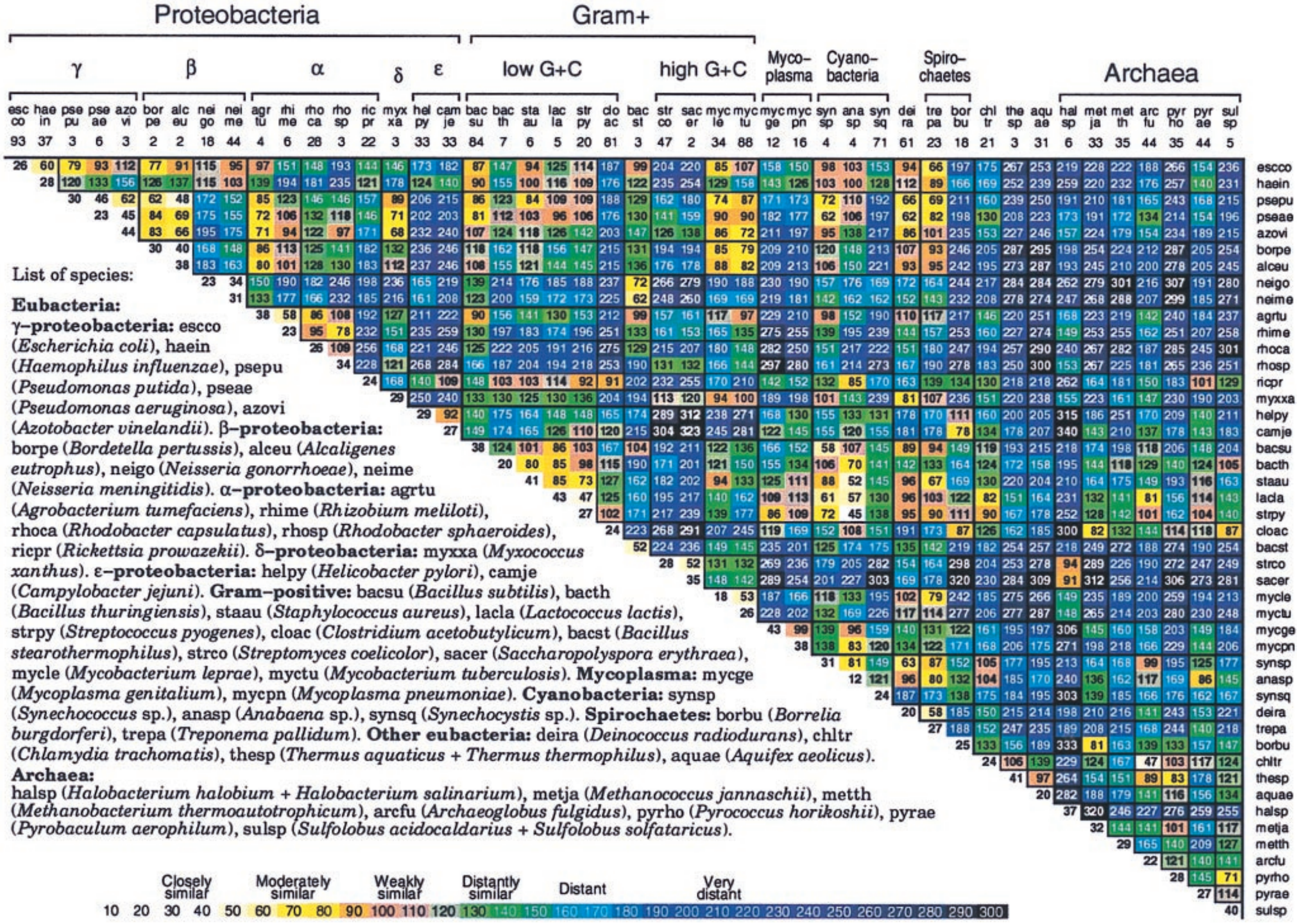
TT/AA is overrepresented in several proteobacteria, in Mycoplasmas, in *Synechocystis* sp., in *Deinococcus radiodurans*, in *A. aeolicus* among prokaryotes, and in insects and worms among eukaryotes. There are no underrepresentations of TT/AA. Overrepresentations of CC/GG include *Synechocystis, B. burgdorferi, A. aeolicus, M. jannaschii, M. thermoautotrophicum*, and *P. horikoshii*. There are no underrepresentations of CC/GG.

δ^* Differences Among Prokaryotic Sequences. Fig. 2 reports average δ^* differences based on multiple disjoint 50-kb contigs among prokaryotic genomic sets, each at least 100 kb in aggregate length and most genome sequence collections exceeding 800 kb. The δ^* differences for all pairs of contigs for every pair of genomes are highly stable with limited variation (cf. refs. 1–6).

(*i*) Within-species δ^* differences (diagonal elements of Fig. 2) range from 12 to 52 (all δ^* differences are multiplied by 1000), and average between species δ^* differences range from 34 to 340.

(*ii*) The rickettsial sequences are grouped with α-proteobacteria, apparently on the basis of rRNA gene comparisons. Is this consistent? The classical α-proteobacterial types are divided into two major subgroups: $\mathscr{A}_1$, including *Rhizobium* spp., that function importantly in nitrogen fixation, and $\mathscr{A}_2$, including *Rhodobacter* spp. and *P. denitrificans*, found predominantly in soil and marine habitats and doing anoxygenic photosynthesis. A tentative third group, $\mathscr{A}_3$, includes the *Rickettsia* and *Ehrlichia* clades (obligate intracellular parasites). Genome signature comparisons indicate drastic discrepancies between the combined groups $\{\mathscr{A}_1, \mathscr{A}_2\}$ and the group $\mathscr{A}_3$. Moreover, the $\mathscr{A}_1$ and $\mathscr{A}_2$ genomes are pervasively of high G+C content ($\geq$60%), whereas $\mathscr{A}_3$ genomes are of low G+C content (<32%). The δ^* differences between *Rickettsia* and classical α-proteobacterial sequences are generally >200, *very distant* (see Fig. 2).

(*iii*) *Sulfolobus* spp. δ^* differences from other prokaryotes. Fig. 2 includes seven archaeal sequence collections consisting of one halobacterial genus (HALSP) (see legends to Figs. 1



Proteobacteria																	Gram+																						Archaea							
γ					β				α					δ	ε		low G+C							high G+C				Myco- plasma		Cyano- bacteria				Spiro- chaetes												
esc co	hae in	pse pu	pse ae	azo vi	bor pe	alc eu	nei go	nei me	agr tu	rhi me	rho ca	rho sp	ric pr	myx xa	hel py	cam je	bac su	bac th	sta au	lac la	str py	clo ac	bac st	str co	sac er	myc le	myc tu	myc ge	myc pn	syn sp	ana sp	syn sq	dei ra	tre pa	bor bu	chl tr	the sp	aqu ae	hal sp	met ja	met th	arc fu	pyr ho	pyr ae	sul sp	
93	37	3	6	3	2	2	18	44	4	6	28	3	22	3	33	33	84	7	6	5	20	81	3	47	2	34	88	12	16	4	4	71	61	23	18	21	3	31	6	33	35	44	35	44	5	
26	60	79	93	112	77	91	115	95	97	151	148	193	144	146	173	182	87	147	94	125	114	187	99	204	220	85	107	158	150	98	103	153	94	66	197	175	267	253	219	228	222	188	266	154	236	escco
	28	120	133	156	126	137	115	103	139	194	181	235	121	178	124	140	90	155	100	116	109	176	122	235	254	129	158	143	126	103	100	128	112	89	166	169	252	239	259	220	232	176	257	140	231	haein
		30	46	62	62	48	172	152	85	123	146	146	157	89	206	215	86	123	84	109	109	188	129	162	180	74	87	171	173	72	110	192	66	69	211	160	239	250	191	210	181	165	243	168	215	psepu
			23	45	84	69	175	155	72	106	132	118	146	71	202	203	81	112	103	96	106	176	130	141	159	90	90	182	177	62	106	197	62	82	198	130	208	223	173	191	172	134	214	154	196	pseae
				44	83	66	195	175	71	94	122	97	171	68	232	240	107	124	118	126	142	203	147	126	138	86	72	211	197	95	138	217	86	101	235	153	227	246	157	224	179	154	234	189	215	azovi
					30	40	168	148	86	113	125	141	182	132	236	246	118	162	118	156	147	215	131	194	194	85	79	209	210	120	148	213	107	93	246	205	287	295	198	254	224	212	287	205	254	borpe
						38	183	163	80	101	128	130	183	112	237	246	108	155	121	144	145	215	136	176	178	88	82	209	213	106	150	221	93	95	242	195	273	287	193	245	210	200	278	205	245	alceu
							23	34	150	190	182	246	198	236	165	219	139	214	176	185	188	237	72	266	279	190	188	230	190	157	176	169	172	164	244	217	284	284	262	279	301	216	307	191	280	neigo
								31	133	177	166	232	185	216	161	208	123	200	159	172	173	225	62	248	260	169	169	219	181	142	162	162	152	143	232	208	278	274	247	268	288	207	299	185	271	neime
									38	58	86	108	192	127	211	222	90	156	141	130	153	212	99	157	161	117	97	229	210	98	152	190	110	117	217	146	220	251	168	223	219	142	240	184	237	agrtu
										23	95	78	232	151	235	259	130	197	183	174	196	251	133	161	153	165	135	275	255	139	195	239	144	157	253	160	227	274	149	253	255	162	251	207	258	rhime
											26	109	256	168	221	246	125	222	205	191	216	275	129	215	207	180	148	282	250	151	217	222	151	180	247	194	257	290	240	267	282	187	285	245	301	rhoca
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													24	168	140	109	148	103	103	114	92	91	202	232	255	170	210	142	152	132	85	170	163	139	134	130	218	218	262	164	181	150	183	101	129	ricpr
														29	250	240	133	130	125	130	136	204	194	113	120	94	100	189	198	101	143	239	81	107	236	151	220	238	155	223	161	147	230	190	203	myxxa
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																27	149	174	165	126	110	120	215	304	323	245	281	122	145	155	120	155	181	178	78	134	178	207	340	143	210	137	178	143	183	camje
																	38	124	101	86	103	167	104	192	211	122	136	166	152	58	107	145	89	94	149	119	193	215	218	174	198	118	206	148	204	bacsu
																		20	80	85	98	115	190	171	201	121	150	155	134	106	70	141	142	133	164	124	172	158	195	144	118	129	140	124	105	bacth
																			41	85	73	127	162	182	202	94	133	125	111	88	52	145	96	67	169	130	220	204	210	164	175	149	193	116	163	staau
																				43	47	125	160	195	217	140	162	109	113	61	57	130	96	103	122	82	151	164	231	132	141	81	156	114	143	lacla
																					27	102	171	217	239	139	177	86	109	72	45	138	95	90	111	90	167	178	252	128	142	101	162	104	140	strpy
																						24	223	268	291	207	245	119	169	152	108	151	191	173	87	126	162	185	300	82	132	144	114	118	87	cloac
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																								28	52	131	132	269	236	179	205	282	154	164	298	204	253	278	94	289	226	190	272	247	249	strco
																									35	148	142	289	254	201	227	303	169	178	320	230	284	309	91	312	256	214	306	273	281	sacer
																										18	53	187	166	118	133	195	102	79	242	185	275	266	149	235	189	200	259	194	213	mycle
																											26	228	202	132	169	226	117	114	277	206	277	287	148	265	214	203	280	230	248	myctu
																												43	99	139	96	159	140	131	122	161	195	197	306	145	160	158	203	149	184	mycge
																													38	138	83	120	134	122	171	168	206	175	271	198	218	166	229	144	206	mycpn
																														31	81	149	63	87	152	105	177	195	213	164	168	99	195	125	177	synsp
																															12	121	96	80	132	104	185	170	240	136	162	117	169	86	145	anasp
																																24	187	173	138	175	184	195	303	139	185	166	176	162	167	synsq
																																	20	58	185	150	215	214	198	210	216	141	243	153	221	deira
																																		27	188	152	247	235	189	208	215	168	244	140	218	trepa
																																			25	133	156	189	333	81	163	139	133	157	147	borbu
																																				24	106	139	229	124	167	47	103	117	124	chltr
																																					41	97	264	154	151	89	83	178	121	thesp
																																						20	282	188	179	141	116	156	134	aquae
																																							37	320	246	227	276	259	255	halsp
																																								32	144	141	101	161	117	metja
																																									29	165	140	209	127	metth
																																										22	121	140	141	arcfu
																																											28	145	71	pyrho
																																												27	114	pyrae
																																													40	sulsp

List of species:

Eubacteria:
γ–proteobacteria: escco (*Escherichia coli*), haein (*Haemophilus influenzae*), psepu (*Pseudomonas putida*), pseae (*Pseudomonas aeruginosa*), azovi (*Azotobacter vinelandii*). **β–proteobacteria:** borpe (*Bordetella pertussis*), alceu (*Alcaligenes eutrophus*), neigo (*Neisseria gonorrhoeae*), neime (*Neisseria meningitidis*). **α–proteobacteria:** agrtu (*Agrobacterium tumefaciens*), rhime (*Rhizobium meliloti*), rhoca (*Rhodobacter capsulatus*), rhosp (*Rhodobacter sphaeroides*), ricpr (*Rickettsia prowazekii*). **δ–proteobacteria:** myxxa (*Myxococcus xanthus*). **ε–proteobacteria:** helpy (*Helicobacter pylori*), camje (*Campylobacter jejuni*). **Gram–positive:** bacsu (*Bacillus subtilis*), bacth (*Bacillus thuringiensis*), staau (*Staphylococcus aureus*), lacla (*Lactococcus lactis*), strpy (*Streptococcus pyogenes*), cloac (*Clostridium acetobutylicum*), bacst (*Bacillus stearothermophilus*), strco (*Streptomyces coelicolor*), sacer (*Saccharopolyspora erythraea*), mycle (*Mycobacterium leprae*), myctu (*Mycobacterium tuberculosis*). **Mycoplasma:** mycge (*Mycoplasma genitalium*), mycpn (*Mycoplasma pneumoniae*). **Cyanobacteria:** synsp (*Synechococcus* sp.), anasp (*Anabaena* sp.), synsq (*Synechocystis* sp.). **Spirochaetes:** borbu (*Borrelia burgdorferi*), trepa (*Treponema pallidum*). **Other eubacteria:** deira (*Deinococcus radiodurans*), chltr (*Chlamydia trachomatis*), thesp (*Thermus aquaticus + Thermus thermophilus*), aquae (*Aquifex aeolicus*).
Archaea:
halsp (*Halobacterium halobium + Halobacterium salinarium*), metja (*Methanococcus jannaschii*), metth (*Methanobacterium thermoautotrophicum*), arcfu (*Archaeoglobus fulgidus*), pyrho (*Pyrococcus horikoshii*), pyrae (*Pyrobaculum aerophilum*), sulsp (*Sulfolobus acidocaldarius + Sulfolobus solfataricus*).

FIG. 2. Average δ* differences within (diagonal entries) and between (nondiagonal entries) prokaryotic DNA sequence samples based on pairwise comparisons of all disjoint nonredundant 50-kb samples available. See also Fig. 5 for 70 prokaryotic species [published as supplemental data on the PNAS web site (www.pnas.org)].

and 2 for full names, and we use the Swiss-Prot abbreviations), two methanogens (METJA, METTH), one "derived" methanogen (ARCFU), and three archaeal thermophiles (PYRHO, PYRAE, SULSP). Thermophiles (exception, PYRHO) tend to be relatively closer to vertebrate eukaryotes than to eubacterial sequences, with δ* differences in the range 100–150 (3, 9), whereas HALSP have generally $\delta^* > 200$. Thus, the highly diverse δ* differences indicate that a consistent description for the archaeal sequences is problematical. We summarize the genomic signature contrasts for *Sulfolobus* in the following array.

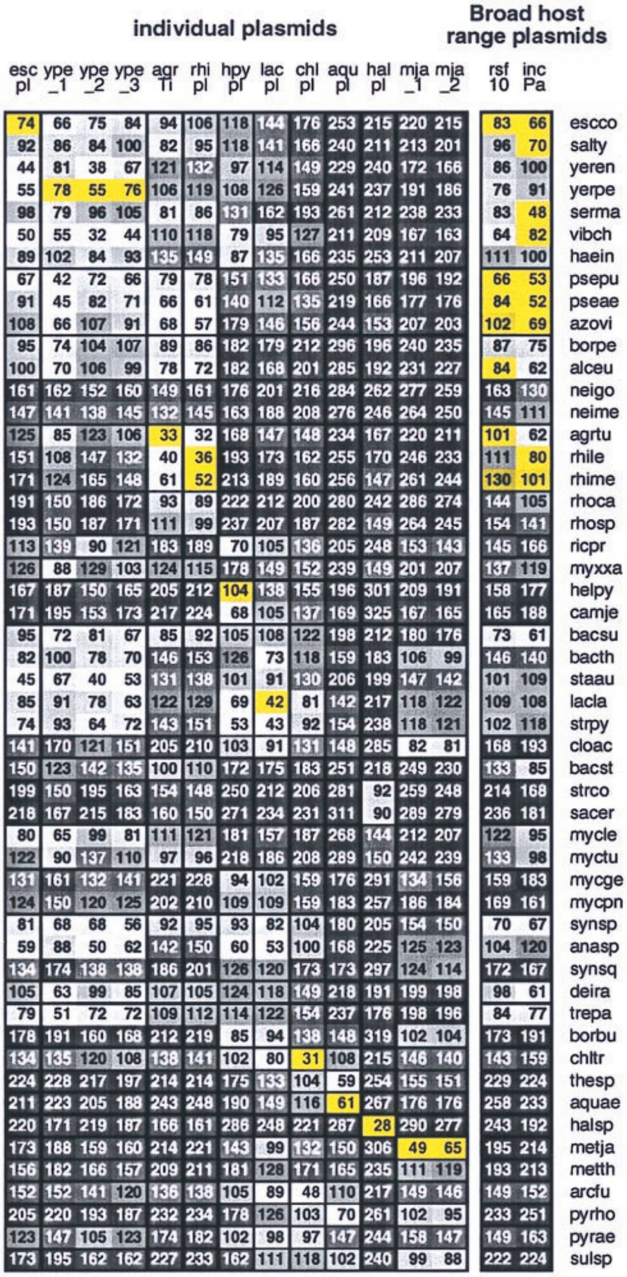
δ* (*Sulfolobus*, *Clostridium*) ≈ 85, *moderately similar*; δ* (*Sulfolobus*, *Rickettsia*, and *Buchnera*) ≈ 125–130, *distantly similar*; δ* (*Sulfolobus*, other thermophilic archaea) ≈ 71–114, *weakly* to *distantly similar*; δ* (*Sulfolobus*, purple proteobacteria and high G+C Gram-positive) ≈ 190–270, *very distant*; δ* (*Sulfolobus*, cyanobacteria) ≈ 145–177, *distant*.

(*iv*) Halobacterial genome sequences are outliers. Intriguingly, with respect to genome signature comparisons, the closest to *Halobacterium* spp. are the *Streptomyces* sequences, *weakly similar*. The δ* differences of *Halobacterium* spp. from other prokaryotes mostly exceed the extreme level of 250.

Genome Compatibility Between Plasmids and Host. Plasmids are genetic mobile elements among bacterial cells, generally laterally transferred by conjugation (on occasion by transduction or transformation). Plasmids carry restriction systems, antibiotic resistance genes, heavy-metal cofactors, Nif (nitrogen fixation) genes, and other contingency functions. Replication of plasmids is largely governed by host machinery. How the genome signature of a plasmid sequence compares with that of its host sequence is assessed for available prokaryotic genomes with completely sequenced plasmids. Fig. 3 reports average δ* differences for each plasmid compared with representatives of 52 prokaryotic genomic collections. Importantly, the δ* differences between each plasmid and its natural host rank among the closest. Thus, the examples of Fig. 3 are consistent with the proposition that plasmids are viable in bacterial hosts only when their genome signatures are sufficiently compatible (*moderately similar*, $55 \leq \delta^* \leq 85$, or at least *weakly similar*, $90 < \delta^* < 115$) with the host genome signature or they can rapidly be made similar.

B. burgdorferi. The complete genome extends 0.911 Mb and contains 17 plasmids, of which 11 have been sequenced from 10 kb to 54 kb in size labeled A to K. The average δ* difference within the *B. burgdorferi* (BORBU) genome is 25 with range 3–66. The δ* differences among all plasmids and compared with a complete set of 50-kb contigs of the host genome range from 42 to 84, clearly *moderately similar* (data not shown). Mutual plasmid sequence δ* differences show *moderate* to *weak similarity*.

Specific plasmids. M. jannaschii, mja. This archaeal genome contains two plasmids of 58 kb (called large) and 16.5 kb (small). Again, we find that the two plasmids are mutually close ($\delta^* = 35$) and each *moderately similar* to the host genome. *Rhizobium* spp., rhi. We have available about 150 kb of nonredundant aggregate sequences from *R. leguminosarum* and about 250 kb of sequence from *R. meliloti*. There is a mammoth plasmid of about 550 kb. Partitioning these sequences into 50-kb contigs yields the average δ* differences given in Fig. 3 of *close similarity*. *Halobacterium* spp., hal. An



individual plasmids													Broad host range plasmids		
esc pl	ype _1	ype _2	ype _3	agr Ti	rhi pl	hpy pl	lac pl	chl pl	aqu pl	hal pl	mja _1	mja _2	rsf 10	inc Pa	
74	66	75	84	94	106	118	144	176	253	215	220	215	83	66	escco
92	86	84	100	82	95	118	141	166	240	211	213	201	96	70	salty
44	81	38	67	121	132	97	114	149	229	240	172	166	86	100	yeren
55	78	55	76	106	119	108	126	159	241	237	191	186	76	91	yerpe
98	79	96	105	81	86	131	162	193	261	212	238	233	83	48	serma
50	55	32	44	110	118	79	95	127	211	209	167	163	64	82	vibch
89	102	84	93	135	149	87	135	166	235	253	211	207	111	100	haein
67	42	72	66	79	78	151	133	166	250	187	196	192	66	53	psepu
91	45	82	71	66	61	140	112	135	219	166	177	176	84	52	pseae
108	66	107	91	68	57	179	146	156	244	153	207	203	102	69	azovi
95	74	104	107	89	86	182	179	212	296	196	240	235	87	75	borpe
100	70	106	99	78	72	182	168	201	285	192	231	227	84	62	alceu
161	162	152	160	149	161	176	201	216	284	262	277	259	163	130	neigo
147	141	138	145	132	145	163	188	208	276	246	264	250	145	111	neime
125	85	123	106	33	32	168	147	148	234	167	220	211	101	62	agrtu
151	108	147	132	40	36	193	173	162	255	170	246	233	111	80	rhile
171	124	165	148	61	52	213	189	160	256	147	261	244	130	101	rhime
191	150	186	172	93	89	222	212	200	280	242	286	274	144	105	rhoca
193	150	187	171	111	99	237	207	187	282	149	264	245	154	141	rhosp
113	139	90	121	183	189	70	105	136	205	248	153	143	145	166	ricpr
126	88	129	103	124	115	178	149	152	239	149	201	207	137	119	myxxa
167	187	150	165	205	212	104	138	155	196	301	209	191	158	177	helpy
171	195	153	173	217	224	68	105	137	169	325	167	165	165	188	camje
95	72	81	67	85	92	105	108	122	198	212	180	176	73	61	bacsu
82	100	78	70	146	153	126	73	118	159	183	106	99	146	140	bacth
45	67	40	53	131	138	101	91	130	206	199	147	142	101	109	staau
85	91	78	63	122	129	69	42	81	142	217	118	122	109	108	lacla
74	93	64	72	143	151	53	43	92	154	238	118	121	102	118	strpy
141	170	121	151	205	210	103	91	131	148	285	82	81	168	193	cloac
150	123	142	135	100	110	172	175	183	251	218	249	230	133	85	bacst
199	150	195	163	154	148	250	212	206	281	92	259	248	214	168	strco
218	167	215	183	160	150	271	234	231	311	90	289	279	236	181	sacer
80	65	99	81	111	121	181	157	187	268	144	212	207	122	95	mycle
122	90	137	110	97	96	218	186	208	289	150	242	239	133	98	myctu
131	161	132	141	221	228	94	102	159	176	291	134	156	159	183	mycge
124	150	120	125	202	210	109	109	159	183	257	186	184	169	161	mycpn
81	68	68	56	92	95	93	82	104	180	205	154	150	70	67	synsp
59	88	50	62	142	150	60	53	100	168	225	125	123	104	120	anasp
134	174	138	138	186	201	126	120	173	173	297	124	114	172	167	synsq
105	63	99	85	107	105	124	118	149	218	191	199	198	98	61	deira
79	51	72	72	109	112	114	122	154	237	176	198	196	84	77	trepa
178	191	160	168	212	219	85	94	138	148	319	102	104	173	191	borbu
134	135	120	108	138	141	102	80	31	108	215	146	140	143	159	chltr
224	228	217	197	214	214	175	133	104	59	254	155	151	229	224	thesp
211	223	205	188	243	248	190	149	116	61	267	176	176	258	233	aquae
220	171	219	187	166	161	286	248	221	287	28	290	277	243	192	halsp
173	188	159	160	214	221	143	99	132	150	306	49	65	195	214	metja
156	182	166	157	209	211	181	128	171	165	235	111	119	193	213	metth
152	152	141	120	136	138	105	89	48	110	217	149	146	149	152	arcfu
205	220	193	187	232	234	178	126	103	70	261	102	95	233	251	pyrho
123	147	105	123	174	182	102	98	97	147	244	158	147	149	163	pyrae
173	195	162	162	227	233	162	111	118	102	240	99	88	222	224	sulsp

FIG. 3. Plasmids are abbreviated as follows: rsf10 (broad-host-range plasmid RSF1010), incPa (Broad host range plasmid Birmingham IncP α), escpl (enterohemorrhagic *E. coli* plasmid pO157), ype_1 (*Yersinia pestis* plasmid pMT-1), ype_2 (*Y. pestis* plasmid pCD1), ype_3 (*Y. pestis* plasmid pPCP1), agrTi (*A. tumefaciens* plasmid Ti), rhipl (*Rhizobium* plasmid pNGR234a), hpypl (*H. pylori* plasmid pHPM186); lacpl (*L. lactis* DPC3147 plasmid pMRC01), chlpl (virulence cluster from *C. trachomatis* plasmid pCHL1), aqupl (*A. aeolicus* plasmid ece1), halpl (*Halobacterium* sp. NRC-1 plasmid), mja_1 (*M. jannaschii* large plasmid), mja_2 (*M. jannaschii* small plasmid). See Fig. 2 for prokaryotic species abbreviations. Additional prokaryotes not included in Fig. 2 are salty (*Salmonella typhimurium*), yeren (*Yersinia enterocolitica*), yerpe (*Yersinia pestis*), serma (*Serratia marcescens*), vibch (*Vibrio cholerae*), and rhile (*Rhizobium leguminosarum*). Yellow background indicates δ^* differences of a plasmid from its confirmed host. See also Fig. 6, which is published as supplemental data on the PNAS web site (www.pnas.org).

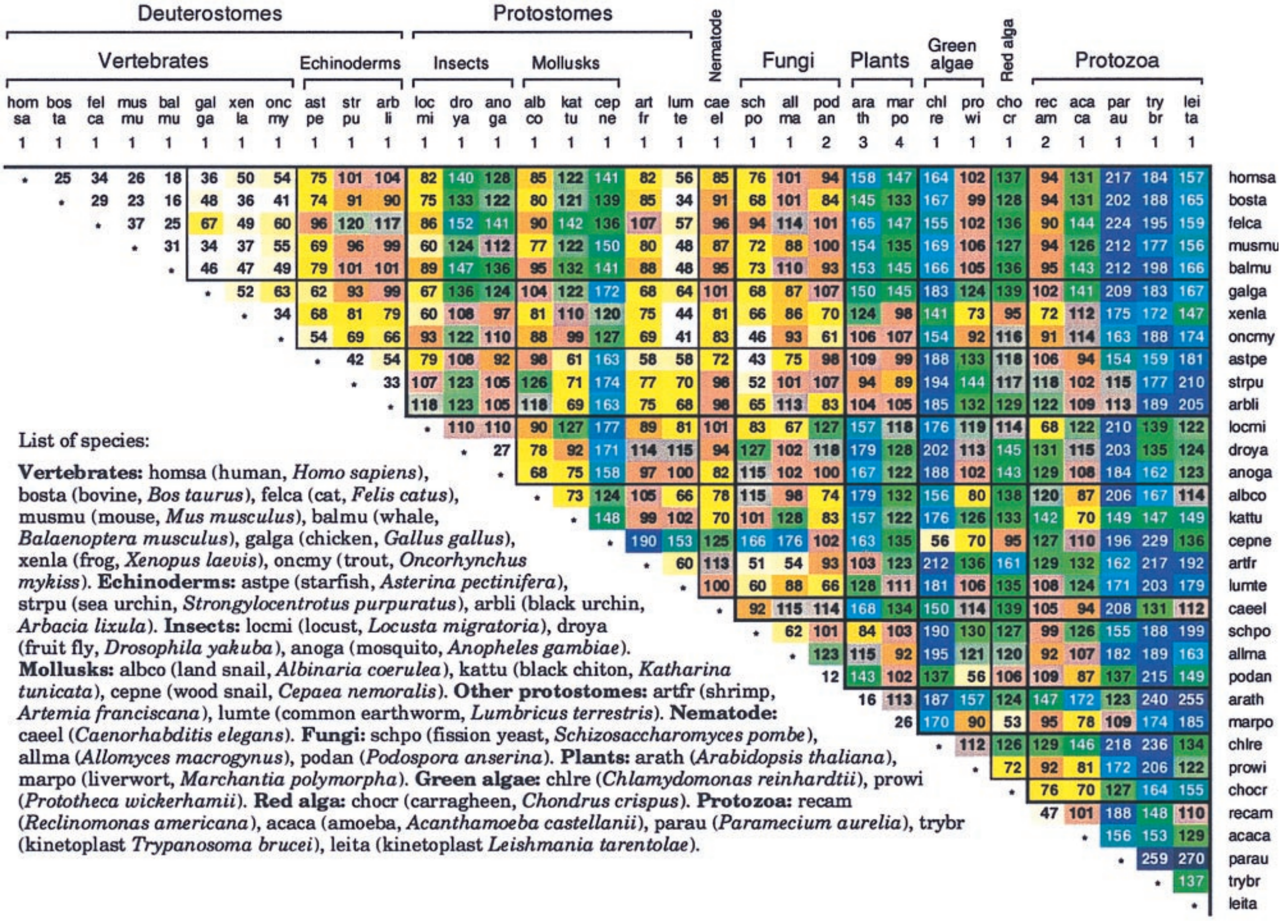
approximately 200-kb plasmid (pNRC100) from *H. halobium* was sequenced. About 100 kb of aggregate sequences from *H. halobium* and *H. salinarium* genomes are also accessible. Fig. 3 reports δ^* differences entailing *close* to *moderate similarity*. *A. tumefaciens*, agr-Ti. Compared to nonplasmid sequences, the classic crown gall tumor plasmid Ti (133 kb available) produces an average δ^* difference of 33 (*closely similar*). *E. coli*, esc. The plasmid O157 of *E. coli*, of length 93 kb, gives δ^* difference of 74 (*moderate similarity*) compared with E. coli. *L. lactis*, lac. This bacterium has available about 250 kb of aggregate genomic sequence and a complete plasmid (MRC01) of length about 60 kb. The δ^* difference of MRC01 to the average 50-kb genome contig is 42 (*closely similar*). *A. aeolicus*, aqu. The complete genome sequence (1.6 Mb) is available. A plasmid contig labeled ece1 has also been sequenced. The δ^* difference is 61 (*moderately similar*). *H pylori*, hpy. The single plasmid (13 kb) of strain 26695 has δ^* difference 104 (*weakly similar*) to the genome. *C. trachomatis*, chl. The plasmid (7.5 kb) compared to the complete genome has a *close* signature difference ($\delta^* = 31$).

Broad-host-range plasmids. Two plasmids that stably replicate in many hosts are RSF1010 and RP4 (13, 14); see legend to Fig. 3. We list next those host prokaryotic species with at least 80 kb of available genome sequence which accept stably these plasmids. RSF1010: *Escherichia coli, Pseudomonas aeruginosa, Pseudomonas putida, Azotobacter vinelandii, Rhizobium meliloti, Agrobacterium tumefaciens*, and *Alcaligenes eutrophus*. RP4: *Serratia marcescens, Azotobacter vinelandii, Pseudomonas aeruginosa, Pseudomonas fluorescens, Escherichia coli, Pseudomonas putida, Rhodospirillum rubrum, Shigella boydii, Salmonella typhimurium, Vibrio cholerae*, and *Rhizobium meliloti*.

The broad-range plasmids are generally *moderately* or *weakly similar* to the other plasmids from proteobacterial genomes. The plasmid from *L. lactis* is *distantly similar* to the broad-range plasmids. The plasmids from *B. burgdorferi, Halobacterium* spp., and *M. jannaschii* are *very distant* from all proteobacterial plasmids. The δ^* differences among *B. burgdorferi* plasmids are mutually *closely* or *moderately similar*, except plasmid G, which is only *weakly similar*. Plasmid G is *close* ($\delta^* = 49$) to the *L. lactis* plasmid, perhaps implying that a low G+C Gram-positive bacterium may be the source of plasmid G. The two broad-range plasmids are mutually *moderately similar* ($\delta^* = 83$). This similarity might mean either that relatively close genome signatures promote plasmid establishment or that the plasmids have acquired their hosts' signatures during long-term residence. Experiments on conjugation can address issues such as specificity vs. wide host range and relevance of size and signature for plasmid compatibility. We interpret the similarities in signature between plasmids and their bacterial hosts as implying that they share much replication and repair machinery, perhaps because the prokaryotic cell is not compartmentalized to the degree that the eukaryotic cell is.

Genomic Signature δ^* Differences Among Mitochondrial Mt Genomes. Fig. 4 details δ^* differences among a broad spectrum of Mt genomes. A succinct summary is given in Table 1.

Mammalian Mt genome signatures appear almost as random samples of each other. Why are the genome signatures among vertebrate Mt sequences significantly more similar than those among the corresponding host nuclear genomic sequences (15)? δ^* differences among organisms putatively reflect variations in replication and/or repair (1–3, 6). The replication machinery for animal Mt DNA apparently varies less than that for host DNA, perhaps because Mt replication is less affected by changes in external environment or developmental programs than is host DNA replication. δ^* differences of Mt from protostomes (e.g., insect, mollusk) versus deuterostomes show δ^* differences mostly in the range 90–115, *weakly similar*. The worm (LUMTE and *C. elegans*) genomes compared with deuterostome Mt are *closely* to *weakly similar*, $\delta^* \approx 41$–101. The fungal Mt sets (excluding *S. cerevisiae*) compared with deuterostome Mt also yield δ^* differences in the range 43–114. The *S. cerevisiae* mtDNA ($\approx$78 kb) composition is an extreme anomaly attested to by the inordinate δ^* differences from all other Mt or nuclear genomes, contributed to by about 100



Deuterostomes											Protostomes																						
Vertebrates								Echinoderms			Insects			Mollusks					Nematode	Fungi			Plants		Green algae		Red alga	Protozoa					
hom sa	bos ta	fel ca	mus mu	bal mu	gal ga	xen la	onc my	ast pe	str pu	arb li	loc mi	dro ya	ano ga	alb co	kat tu	cep ne	art fr	lum te	cae el	sch po	all ma	pod an	ara th	mar po	chl re	pro wi	cho cr	rec am	aca ca	par au	try br	lei ta	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	3	4	1	1	1	2	1	1	1	1	
•	25	34	26	18	36	50	54	75	101	104	82	140	128	85	122	141	82	56	85	76	101	94	158	147	164	102	137	94	131	217	184	157	homsa
	•	29	23	16	48	36	41	74	91	90	75	133	122	80	121	139	85	34	91	68	101	84	145	133	167	99	128	94	131	202	188	165	bosta
		•	37	25	67	49	60	96	120	117	86	152	141	90	142	136	107	57	96	94	114	101	165	147	155	102	136	90	144	224	195	159	felca
			•	31	34	37	55	69	96	99	60	124	112	77	122	150	80	48	87	72	88	100	154	135	169	106	127	94	126	212	177	156	musmu
				•	46	47	49	79	101	101	89	147	136	95	132	141	88	48	95	73	110	93	153	145	166	105	136	95	143	212	198	166	balmu
					•	52	63	62	93	99	67	136	124	104	122	172	68	64	101	68	87	107	150	145	183	124	139	102	141	209	183	167	galga
						•	34	68	81	79	60	108	97	81	110	120	75	44	81	66	86	70	124	98	141	73	95	72	112	175	172	147	xenla
							•	54	69	66	93	122	110	88	99	127	69	41	83	46	93	61	106	107	154	92	116	91	114	163	188	174	oncmy
								•	42	54	79	108	92	98	61	163	58	58	72	43	75	98	109	99	188	133	118	106	94	154	159	181	astpe
									•	33	107	123	105	126	71	174	77	70	98	52	101	107	94	89	194	144	117	118	102	115	177	210	strpu
										•	118	123	105	118	69	163	75	68	98	65	113	83	104	105	185	132	129	122	109	113	189	205	arbli
											•	110	110	90	127	177	89	81	101	83	67	127	157	118	176	119	114	68	122	210	139	122	locmi
												•	27	78	92	171	114	115	94	127	102	118	179	128	202	113	145	131	115	203	135	124	droya
													•	68	75	158	97	100	82	115	102	100	167	122	188	102	143	129	108	184	162	123	anoga
														•	73	124	105	66	78	115	98	74	179	132	156	80	138	120	87	206	167	114	albco
															•	148	99	102	70	101	128	83	157	122	176	126	133	142	70	149	147	149	kattu
																•	190	153	125	166	176	102	163	135	56	70	95	127	110	196	229	136	cepne
																	•	60	113	51	54	93	103	123	212	136	161	129	132	162	217	192	artfr
																		•	100	60	88	66	128	111	181	106	135	108	124	171	203	179	lumte
																			•	92	115	114	168	134	150	114	139	105	94	208	131	112	caeel
																				•	62	101	84	103	190	130	127	99	126	155	188	199	schpo
																					•	123	115	92	195	121	120	92	107	182	189	163	allma
																						12	143	102	137	56	106	109	87	137	215	149	podan
																							16	113	187	157	124	147	172	123	240	255	arath
																								26	170	90	53	95	78	109	174	185	marpo
																									•	112	126	129	146	218	236	134	chlre
																										•	72	92	81	172	206	122	prowi
																											•	76	70	127	164	155	chocr
																												47	101	188	148	110	recam
																													•	156	153	129	acaca
																														•	259	270	parau
																															•	137	trybr
																																•	leita

List of species:

Vertebrates: homsa (human, *Homo sapiens*), bosta (bovine, *Bos taurus*), felca (cat, *Felis catus*), musmu (mouse, *Mus musculus*), balmu (whale, *Balaenoptera musculus*), galga (chicken, *Gallus gallus*), xenla (frog, *Xenopus laevis*), oncmy (trout, *Oncorhynchus mykiss*). **Echinoderms:** astpe (starfish, *Asterina pectinifera*), strpu (sea urchin, *Strongylocentrotus purpuratus*), arbli (black urchin, *Arbacia lixula*). **Insects:** locmi (locust, *Locusta migratoria*), droya (fruit fly, *Drosophila yakuba*), anoga (mosquito, *Anopheles gambiae*). **Mollusks:** albco (land snail, *Albinaria coerulea*), kattu (black chiton, *Katharina tunicata*), cepne (wood snail, *Cepaea nemoralis*). **Other protostomes:** artfr (shrimp, *Artemia franciscana*), lumte (common earthworm, *Lumbricus terrestris*). **Nematode:** caeel (*Caenorhabditis elegans*). **Fungi:** schpo (fission yeast, *Schizosaccharomyces pombe*), allma (*Allomyces macrogynus*), podan (*Podospora anserina*). **Plants:** arath (*Arabidopsis thaliana*), marpo (liverwort, *Marchantia polymorpha*). **Green algae:** chlre (*Chlamydomonas reinhardtii*), prowi (*Prototheca wickerhamii*). **Red alga:** chocr (carragheen, *Chondrus crispus*). **Protozoa:** recam (*Reclinomonas americana*), acaca (amoeba, *Acanthamoeba castellanii*), parau (*Paramecium aurelia*), trybr (kinetoplast *Trypanosoma brucei*), leita (kinetoplast *Leishmania tarentolae*).

FIG. 4. Average δ* differences among complete Mt genomes based on a single sequence sample for genomes of <60 kb and multiple samples of about 50 kb for larger genomes.

G+C-rich clusters, each about 50 to 100 bp long, separated by A+T-rich spacers and numerous transposable elements.

The two plant Mt genomes (ARATH, MARPO, see Fig. 4), of about 150-kb length, compared with animal Mt are *weakly similar* to *distant*. The green alga CHLRE Mt is *very distant* from most other Mt sequences (δ* > 180). The other two algal Mt sequences (PROWI, CHOCR) are generally *weakly similar* to nonprotist Mt sequences. The protist Mt divide into two subgroups, P1 = (RECAM, ACACA) and P2 = (PARAU, TRYBR, LEITA). The RECAM Mt genome signature compared with deuterostome Mt is *weakly* to *distantly similar*. ACACA is a bit farther (δ* ≈ 94–144). The protist group P2 are mostly *distant* to *very distant* from animal Mt sequences. The plant *A. thaliana* Mt sequence is *distant* from mammalian Mt sequences, possibly suggesting a polyphyletic ancestry separating metazoan, plant, and protist Mt.

Animal Mt sequences show significant underrepresentations of CG dinucleotides, $\rho^*_{\mathrm{CG}} \approx 0.40–0.60$ (10), about to the same extent as occurs in vertebrate nuclear genomic sequences. Virtually all animal Mt maintain normal representations of TA dinucleotides, whereas the corresponding nuclear DNAs predominantly have TA in low relative abundance, suggesting that mtDNA may be thermodynamically less stable than nuclear DNA because the dinucleotide TA has the lowest stacking energies compared with all other base steps. The fungal Mt of *S. pombe* has $\rho^*_{\mathrm{CG}} \approx 0.54$, typical of animal Mt. However, the *Podospora anserina* fungal Mt genome has ρ^*_{CG} in the normal range. The Mt genome of *A. thaliana* has $\rho^*_{\mathrm{CG}} = 0.73$, significantly low. The single persistently high ρ* value occurs for $\rho^*_{\mathrm{CC/GG}} \geq 1.30$ in animal and fungal Mt sequences. δ* differences between species parallel the corresponding nuclear δ* differences, despite large differences between Mt and corresponding host nuclear signatures (15).

Discussion Prokaryotic molecular taxonomy heretofore has been derived predominantly from sequence comparisons among rRNA genes. There are many uncertainties and controversies regarding divisions among prokaryotes (for recent reviews, see refs. 16 and 17). Protein sequence comparisons and associated phylogenetic tree constructions are even more conflicting relative to evolutionary relationships (18, 19).

Table 1. Summary of δ* differences of Mt genomes

Comparison	δ*	Description
Among mammalian Mt	<40	*Very close*
Among all vertebrates including birds, frogs, fish	<67	*Mainly close*
Vertebrates vs. nonvertebrate deuterostomes	55–120	*Moderate* to *weak similarity*
Deuterostomes vs. protostomes	Mostly 34–152	*Moderate* to *distant similarity*
Fungi vs. animals	Mostly 43–128	*Moderate* to *weak similarity*
Plants vs. (animals and fungi)	84–179	*Weak similarity* to *distant*
C. reinhardtii vs. animals	Mostly 141–212	*Distant* to *very distant*

Conventional methods of phylogenetic reconstruction from sequence information employ only similarity or dissimilarity assessments of aligned homologous genes or regions. Some difficulties intrinsic to this approach compared to the use of genome signature include the following: (*i*) Alignments of distantly related long sequences (e.g., complete genomes) are generally not feasible for various reasons, including chromosomal rearrangements, whereas signature comparisons do not depend on alignments. (*ii*) Different phylogenetic reconstructions (trees) may result for the same set of organisms based on analysis of different protein, gene, or noncoding sequences. Attempts to overcome these conflicts by "averaging" over many proteins are problematic because of biases in species sampling, effects of lateral transfer, complications of gene duplications, and inadequacies and artifacts of phylogenetic methods. As the signature has remarkably low variance throughout the genome, a tree based on δ^* differences is independent of which genome segments of 50 kb (or longer) is used in its construction. (*iii*) Chimeric origins and lateral transfer between distantly related organisms complicate alignment-based phylogenies. Signature comparisons are unaffected by these factors. On the other hand, alignment-based comparisons may provide information on individual gene origins that the signature method does not. (*iv*) Tree construction derived from aligned sequences cannot be applied to organisms for which similar gene sequences are largely unavailable (e.g., for bacteriophages, diverse eukaryotic viruses). (*v*) That "lateral transfer" is pervasive among prokaryotic genomes is now widely appreciated. Vectors for lateral transfer include exogenous transposons, hitchhiking on plasmids and/or phages, movement via episomes, and cell fusions.

What possible reasons and mechanisms can account for the qualitative parallelism between the evolutionary development of host nuclear genomes and the development of Mt organelle genomes despite the pronounced difference between the Mt and host nuclear genome signatures? The Mt and nuclear genomes for animal and fungal organisms use independent DNA polymerase machinery (e.g., γ vs. α, ε, and δ subunits in mammals). Also, the methods of replication and the nature of the replication origins are fundamentally different. Explicitly, the animal and fungal Mt transcription-primed replication machinery is distinctive in that most of the heavy strand is synthesized first and the light strand subsequently, whereas the nuclear genomes are replicated bidirectionally from multiple origins. There appears to be no DNA excision repair mechanism to deal with cyclobutane dimers in the Mt, and no repair of bulky lesions (20). Mt DNAs in animals and fungi show elevated levels of single- and double-strand breaks, mismatches, and generally corrupted base pairings, probably due to a paucity of abasic site correction facilities and mismatch repair capacity in Mt genomes (21). Moreover, repair may be less urgent for Mt activity because each cell has many mitochondria (hundreds or thousands) and a modicum of impaired organelles may not significantly curtail energy production. We, nevertheless, propose that Mt genomes retain signatures close to those of their repair-competent prokaryotic ancestor.

The contrast between plasmids (which track host genomic signatures) and mitochondria (which do not) is sharp. The similar signatures of plasmids and hosts may have two bases: (*i*) As we have postulated for genome fusions (8), perhaps a plasmid whose signature is too different from that of the host will not be accepted by it. This would prescribe a maximum possible signature deviation. The largest δ^* difference observed in our survey is *weakly similar* (or about the distance from human to sea urchin). (*ii*) During the plasmid's residence in its current host, the same pressures that homogenize the signature throughout the chromosome will also drive the plasmid's signature towards that of the host. Such amelioration has been postulated for the G+C content of laterally transferred DNA (22). We suspect that the signature should ameliorate even more rapidly, for both plasmids and laterally transferred chromosomal segments. Whereas most successful gene transfer between lineages is very likely intraspecific, an appreciable amount of transfer among distantly related bacteria seems to have accumulated over time (22, 23). Despite such transfer, the signatures currently observed are almost invariant throughout the genome. Some means based on codon usage biases for ascertainments of laterally transferred genes in bacterial organisms are set forth in ref. 23.

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1. Karlin, S. & Burge, C. (1995) *Trends Genet.* **11,** 283–290.
2. Blaisdell, B. E., Campbell, A. M. & Karlin, S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5854–5859.
3. Karlin, S., Mrázek, J. & Campbell, A. M. (1997) *J. Bacteriol.* **179,** 3899–3913.
4. Josse, J., Kaiser, A. D. & Kornberg, A. (1961) *J. Biol. Chem.* **263,** 864–875.
5. Russell, G. J., Walker, P. M., Elton, R. A. & Subak-Sharpe, J. H. (1976) *J. Mol. Biol.* **108,** 1–23.
6. Karlin, S. (1998) *Curr. Opin. Microbiol.* **1,** 598–610.
7. Karlin, S. & Mrázek, J. (1996) *J. Mol. Biol.* **262,** 459–472.
8. Karlin, S., Brocchieri, L., Mrázek, J., Campbell, A. M. & Spormann, A. M. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 9190–9195.
9. Karlin, S., Campbell, A. M. & Mrázek, J. (1998) *Annu. Rev. Genet.* **32,** 185–225.
10. Cardon, L. R., Burge, C., Clayton, D. A. & Karlin, S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3799–3803.
11. Karlin, S., Doerfler, W. & Cardon, L. R. (1994) *J. Virol.* **68,** 2889–2897.
12. Krieg, A. M., Yi, A.-K., Schorr, J. & Davis, H. L. (1998) *Trends Microbiol.* **6,** 23–27.
13. Olsen, R. H. & Shipley, P. (1973) *J. Bacteriol.* **113,** 772–780.
14. Bagdasarian, M., Lurz, R., Ruckert, B., Franklin, F. C., Bagdasarian, M. M., Frey, J. & Timmis, K. N. (1981) *Gene* **16,** 237–247.
15. Karlin, S. & Mrázek, J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 10227–10232.
16. Brown, J. R. & Doolittle, W. R. (1997) *Microbiol. Rev.* **61,** 456–502.
17. Gupta, R. S. (1998) *Microbiol. Mol. Biol. Rev.* **62,** 1435–1491.
18. Gupta, R. S. & Golding, G. B. (1996) *Trends Biochem. Sci.* **21,** 166–171.
19. Budin, K. & Philippe, H. (1998) *Mol. Biol. Evol.* **15,** 943–956.
20. Shadel, G. S. & Clayton, D. A. (1997) *Annu. Rev. Biochem.* **66,** 409–435.
21. Yakes, F. M. & Van Houten, B. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 514–519.
22. Lawrence, J. G. & Ochman, H. (1997) *J. Mol. Evol.* **44,** 383–397.
23. Karlin, S., Mrázek, J. & Campbell, A. M. (1998) *Mol. Microbiol.* **29,** 1341–1355.