Classification: Evolution and Genetics

Gene-centric intra- and inter-clade recombination in a context of *Escherichia coli* subpopulations

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Running Head: Fine-scale Map of Genome-wide Recombination

ABSTRACT（250 words）

Recombination is one of the most important mechanisms of prokaryotic species evolution but its exact roles are still in debate. Here we try to infer genome-wide recombination events within a species utilizing a dataset of 104 complete genomes of *Escherichia coli* from diverse origins, among which 45 from world-wide animal-hosts are in-house sequenced using SMRT (single-molecular real time) technology. Two major clades are identified based on evidences of ecological and physiological characteristics, as well as distinct genomic features implying scarce inter-clade genetic exchange. By comparing the synteny of identical fragments genome-widely searched for each genome pair, we achieve a fine-scale map of recombination within the population. The recombination is rather extensive within clade, which is able to break linkages between genes but does not interrupt core genome framework and primary metabolic portfolios possibly due to natural selection for physiological compatibility and ecological fitness. Meanwhile, the recombination between clades declines drastically as the phylogenetic distance increases, generally 10-fold reduced than those of the intra-clade, which establishes genetic barrier between clades. These empirical data of recombination suggest its critical role in the early stage of speciation, where recombination rate differs according to phylogentic distance. The extensive intra-clade recombination coheres sister strains into a quasi-sexual group and optimizes genes or alleles to streamline physiological activities, whereas shapely declined inter-clade recombination split the population into clades adaptive to divergent ecological niches.

Significance Statement（120 words）

Roles of recombination in species evolution have been debated for decades due to difficulties in inferring recombination events during the early stage of speciation, especially when recombination is always complicated by frequent gene transfer events of bacterial genomes. Based on 104 high-quality complete *E. coli* genomes, we infer gene-centric dynamics of recombination in the formation of two *E. coli* clades or subpopulations, and recombination is found to be rather intensive in a within-clade fashion, which forces them to be quasi-sexual. The recombination events can be mapped among individual genomes in the context of genes and their variations; decreased between-clade and increased intra-clade

recombination engender a genetic barrier that further encourages clade-specific secondary metabolic portfolios for better environmental adaptation. Recombination is thus a major force that accelerates bacterial evolution to fit ecological diversity.

Main Text

Introduction

Roles of recombination in bacterial evolution have been debated for decades (1, 2). Asexual bacteria are believed to rarely recombine their genomes when passing genetic information to progenies, and a cluster of genes is capable of taking over the entire population if they happen to be linked with an advantageous gene through genetic hitchhiking, leading to an effect of genome-wide selective sweep as proposed in an “ecotype model”(3, 4). The theory is supported by a recent time-series metagenomics research (5), whereas other studies reveal the counterpart where recombination breaks the linkage and leaves only the adaptive gene to sweep a population (6, 7). The issue comes that to what extent recombination actually breaks such genetic linkages and undermines the theory of genome sweep. Another issue relevant to recombination is its contradictory roles in speciation, where recombination produces inconsistent signals in clonal phylogenies (8) in one hand and hijacks similar strains into a separating subpopulation (1, 9) in the other. Recent studies have postulated extensive recombination in bacterial populations even to a quasi-sexual status(6). Will extensive recombination interrupt primary metabolic portfolios or genome organization frameworks (GOF) (10), and lead to puzzled or discordant phylogenies? These issues can be readily settled by resolving recombination events in a genome-scale and at single gene level within a bacterial population where subpopulations are recently diverged, making a fine-scale and panoramic display of the recombination dynamics in early speciation. However, it is still unachievable due to the difficulties in inferring recombination events between closely related strains (11).

Here, we investigate the species *E. coli*, an excellent model for studying genotype-phenotype relationships and molecular evolution mechanisms due to its collective knowledge, ecological diversity, and availability of high-quality genomes and their annotations. Pangenomic studies have revealed great genome variability and intensive recombination in *E. coli*, which underlie discordance between phylogenies of various orthologous genes (12). To generate recombination dynamics genome-wide and in a fine-scale, we start with 45 in-house genome assemblies from animal hosts, using SMRT (Single-molecule real-time

82 sequencing) technology, and also put together a collection of 104 complete genome sequences of strains
83 from diverse geographic and host range from public databases. Based on genome-based phylogenies
84 coupled with experimentation and detailed genetic analysis, we further classify *E. coli* isolates into two ma-
85 jor clades or subpopulations, *Vigorous* for its fast movement and *Sluggish* for its slow movement, abbrevi-
86 ated as Vig and Slu. Taking advantage of our complete genome assemblies, as well as their precise
87 synteny of identical fragments and genes, we are able to infer recombination events for each genome pair
88 and to calculate the mobility (transfer among isolates within and across clades) of each gene, which are
89 used to address: (1) In what degree and intensity does recombination happen within a species and its
90 subpopulations? (2) What are molecular and physiological bases of the early separation of clades? (3)
91 Does recombination disrupt GOF and metabolic portfolios of a host strain?
92

93 **Results**

94 Our study begins with a thorough collection of 202 *E. coli* strains of animal hosts with broad diver-
95 sity in geography, climate, and host range by field scientists, and the strains have been served as repre-
96 sentation of the species for detailed taxonomic characterization (13). Of this collection, 45 isolates which
97 represent maximal genetic heterogeneity and biodiversity in terms of host diet, geographic distribution
98 (Fig. S1) and MLST clustering (Fig. S2), are selected for genome sequencing with both Illunima and Pac-
99 Bio RSII platform; and all genomes are finished to a single circular chromosome.
100 For sequence analysis, we retrieve 59 complete genomes of human-host strains together with 7
101 draft genomes of environmental strains (14) from the public databases. Based on the 111 genomes, we
102 extract the core genome of *E. coli*, which has collectively 1,095 genes in a total length of 1.05 Mb. The
103 maximal-likelihood phylogeny based on the core genome indicates clear separation between host-related
104 and environmental strains, whereas human- and animal-host strains appear entangled (Fig.1). Most host-
105 related strains cluster into two major clades; the larger one contains 56 strains of phylotype A and B1,
106 whereas the smaller one is composed of 31 phylotype B2 strains. The rest 17 strains, mainly in phylotype
107 D and E, form several minor clades and are more closely positioned to the environment strains. Overall,
108 this clustering pattern is largely in congruence with their phylotypes and previous reports (15, 16). As for
109 the two major clades, although they both contain commensals and pathogens and show overlaps in a
110 wide range of hosts and geographic distributions, they still exhibit skewed distributions in some ecological

111 and clinical niches. The larger clade includes strains that prefer tropics, carnivorous hosts, and occasion-
112 ally lead to pandemic infections, whereas strains in the smaller clade show the opposite preferences as
113 cold climate, herbivorous host, and often cause extra-intestinal and antibiotic-resistant infections. We fur-
114 ther explore the reasons underlying these ecological distinctions by performing physiological experiments
115 with our collection strains in the two clades. The strains in the larger clade show higher growth rate, espe-
116 cially in higher temperature, apparent vulnerability and poorer survival rates at heat stress due to faster
117 growth, rapid response and motility to amino acid chemotaxis, as well as slower growth in polysaccharides
118 carbohydrate sources when compared to the smaller clade (Fig.S3). We subsequently name the two
119 clades as the Vig and the Slu clades, respectively.

120 We examine genetic bases of the phenotypical distinctions between Vig and Slu in their genomes.
121 First, we find apparent differences between the two clades in their profiles of dispensable genes based in
122 a neighbor-joining tree (Fig. S4). By using two-sample Kolmogorov-Smirnov test to all dispensable genes,
123 we identify 279 and 336 orthologs that are significantly enriched in Vig and Slu, respectively (Table S2).
124 Half of these clade-associated-genes (CAGs) are metabolic genes according to the COG categories, and
125 as expected (Fig.S5A), the Vig clade enriches more genes related to motility and metabolism of purine
126 and amino acid than the Slu clade. Strains of the same clade share common patterns in dispensable met-
127 abolic genes regardless of host diet preferences (Fig.S5B). We further predict nutrient consumption and
128 fermentation products by constructing the genome-scale models (GSMs) of metabolism for each strain *in*
129 *silico* (17, 18), the two clades are largely separated by characteristic substrates and products, albeit a few
130 confusions (Fig. 1B). Given a complete rich media which contains 16279 compounds, GSMs predict that
131 our stains consume or produce 102 of these compounds, and 82 show differences between strains. A total
132 of 12 compounds show significant difference (p<0.01, Wilcoxon test) between the two clades, i.e., the Slu
133 strains uptake more plant-origin nutrients of dulcose, maltose, and cellobiose, whereas the Vig strains up-
134 take more taurine and xylose. The Vig strains produce more formate, glycerol, and L-rhamnose, as com-
135 pared to the Slu strains that produce more lactate and L-proline and are more active in xanthine metabo-
136 lism. For dispensable virulence genes, the Vig pathogens are rich in T3SS and other secretion systems
137 and the lethal Shiga-like toxins (Stx) (19), whereas the Slu pathogens have more genes for adhesion, in-
138 vasion and Extended-Spectrum Beta Lactamases (ESBLs) that possibly facilitate their extra-intestinal in-
139 fections and antibiotic-resistance (Fig.S5C,S5D). Next, we compare sequence variances between
140 orthologs shared by the two clades. In the core genome, 126 and 227 non-synonymous polymorphisms in

141 97 and 168 genes show specificity for the Vig and Slu clades, respectively, which scatter along the core
142 genome (Fig.S6A, Table S3). Half of the genes that contain these clade-associated variations (CAVs) are
143 in the COG category of metabolism, which also underpin the metabolic distinctions between the two
144 clades (Fig.S6B). Furthermore, homologues of the core genes involved in the metabolic pathways of
145 amino acids and carbohydrates show a clear separation in their maximal-likelihood phylogeny when con-
146 catenated (Fig. S7). Dispensable orthologs with high prevalence (present in > 80% of the 104 strains) also
147 show notable larger sequence distance in paired homologs between-clade than within-clade (Fig. S8).
148 These differences in sequence distance suggest that each clade utilizes their own preferred alleles of both
149 the core and dispensable genes, which are not readily traded between clades.
150 The obvious genetic differences between the clades suggest they exchange genetic material
151 scarcely. We thus infer genome-wide recombination for each genome pair to see if recombination de-
152 creases sharply and shows signals of genetic separation between the two clades. Taking advantage of
153 our complete genomes, we are able to infer recombination events by comparing synteny of aligned identi-
154 cal fragments. These fragments if moved to other locations are recognized as authentic recombinant frag-
155 ments. Our result shows that extent of intra-clade recombination is rather extensive than that of inter-clade
156 (Fig. 2A), in accordance with a previous report of less samples(20). For intra-clade genome pairs, the total
157 lengths of recombinant fragments range 0.3Mb – 2Mb, and in some cases even approaching half of the
158 genome, whereas those of inter-clade pairs are 10-fold shorter and never exceed 250kb (Fig. 2B). The
159 intensity of intra-clade recombination exhibit almost the same extent even between commensal and patho-
160 genic strains (Fig. 2B inlet), which implies that virulence genes can be freely transferred between com-
161 mensals and pathogens of the same clade and makes efforts to distinguish them extremely difficult (21).
162 Not only the intensity of recombination shows a clear cut-off between inter- and intra-clade recombination,
163 but also logarithmically correlated to phylogenetic distance based on core genome polymorphism (Fig.2C)
164 as previously estimated (22). Seemingly, the numbers of fragments that represent recombination fre-
165 quency are much greater in intra-clade pairs than inter-clade pairs (Fig. 2D) and correlate with phyloge-
166 netic distance as well (Fig. 2E), except in case of very close strains where recombination frequency can
167 be significantly underestimated due to the overlapping of recombinant fragments. The average size of re-
168 combinant fragments is also much longer in intra-clade genome pairs than what of inter-clade (Fig. 2F)
169 and correlate with phylogenetic distance (Fig. 2G). Since restriction-modification system has been demon-

170 strated to control recombination between species (23), we test its role in our samples with the DNA meth-
171 ylation information obtained from PacBio platform. However, the result shows no clade-specific restriction
172 motif, although there are many strain-specific methylated motifs and a common motif of GATC in all *E. coli*
173 strains (Fig. S9).

174 The majority of recombinant fragments are in length of 3kb which fits in the common size of bacte-
175 rial operons, even some super-fragments can approach 2Mb (Fig. 3A) as long as reported in other species
176 (24, 25). As so efficient and frequent the recombination is in exchanging genetic materials especially
177 within clade, we further examine its influence on the organization and metabolic scheme of the recipient
178 genome. Firstly, we calculate the mobility of each single gene as its normalized frequency of being recom-
179 bined. The result indicates a broad range of gene mobility from 0 to 100% and a clear negative correlation
180 to prevalence, i.e., core genes which present in all strains are rather stable and seldom recombined,
181 whereas dispensable genes, especially those only present in few strains, are actively transferred between
182 strains (Fig. 3B). This is in accordance with the concept of Genome Organization Framework (GOF) (10),
183 where core genes constitute a stable genome framework, leaving the intervals filled with mobile variable
184 genes. Next, we examine the mobility of the clade-characteristic CAGs which show comparable overall
185 mobility as other dispensable genes of similar prevalence (Fig. 3B), but differ their mobility in the two
186 clades: Vig-CAGs are much more stable in Vig strains than in Slu strains, and those of Slu show opposite
187 mobility in the two clades (Fig. 3C). As for the core genes containing CAVs, they are all rarely recombined,
188 but still a little more stable in corresponding clades although the difference is not significant (Fig. 3D). The
189 stability of core genes as well as CAGs in corresponding clades suggest that the recombination has little
190 overall influences on genes with critical adaptive functions. Thus the extensive recombination does not
191 disturb the framework of genome and adaptive metabolic scheme heavily, possibly due to strong negative
192 selections for any changes that impair the fitness of host cell, according to the complexity hypothesis and
193 cellular evolution(26).
194

195 **Discussion**

196 Our study is based on a dataset of high-quality complete genomes as well as physiological features
197 experimentally acquired for the representative collection of *E. coli* strains. Two major clades of the popu-
198 lation, Vig and Slu, show distinct ecological and clinical features due to their physiological differences and
199 clade-featured metabolic profile. The phenotypes of the two clades are genetically underpinned by their

differences in genome content and alleles they used for orthologous which make them adaptive to respective ecological niches. The thorough inference of recombination events for each pair of genomes indicates that recombination is rather frequent and intensive between within-clade strains but logarithmically reduced with phylogenetic distance, leading to genetic separation between clades. The extensive recombination, although keep cutting-and-pasting new genetic materials until they reach assortive genome locations, do not untidy GOFs and adaptive metabolic portfolios.

One of our findings is to quantitate the intensity of genetic recombination occurring within and across the *E. coli* clades we have defined, which is not easy due to weak signals of recombined sequences intrinsic to draft assemblies (11, 27). For a long time, recombination has been acknowledged to be very rare in asexual bacterial species, on which the theory of genome sweep has been established and genes adjacent to advantageous alleles may achieve high prevalence through genetic hitchhiking. Recent studies have questioned the theory and proposed bacteria as quasi-sexual populations where adaptive genes instead of genome sweep the population driven by nature selection (6, 7). Our data, providing exactly count on each recombination event for genome pairs, directly verify a high intra-clade recombination rate and quasi-sexual status of microbial populations.

The poor inter-clade recombination rate and its small recombinant fragment size suggest a mechanistic barrier in favor of speciation (28). In simulations of evolution model, given suitable mutation rate and enough population size, even without other involvement of nature selection or geographic separation, populations can diverge neutrally only if recombination rate depends on homolog distance (similar to phylogeny distance in our work) (29). In other words, if individuals in a population recombine more intensively with closer relatives, the disparity in recombination rate would by itself homogenize strains with similar genome sequences into a clade and build genetic barriers to others (28). As a result, the disparity of recombination intensity may consistently generate new clades; some of them are deemed to be adaptive or specialized to ecological niches and selected by nature and others may just drift on and off the edge. This is precisely the scenario of the Vig and Slu clade separation in our study, and such scenarios have been published in other bacteria , archaea (7, 30-33) and eukaryotes (34). However, molecular mechanism of the phylogeny-restricted recombination have yet to be described. Previous studies have proposed roles of geographic isolation, diversity of CRISPR-Cas system (35), DNA uptake signal sequences (36), and incompatible transfer mechanisms due to pili (37) for the mechanisms. Recently, high-efficiency recombination mechanisms, such as co-evolving phage and distributive conjugal transfer have been reported, which

230 allows DNA fragments over 100kb in a single recombination event to happen but such a function deterio-
231 rates between remote relatives (20, 38). All these mechanisms with various transfer capacities and host
232 range enable strains to utilize suitable means in exchanging genetic material according to phylogenetic
233 distance of relatives.

234 The assessment of mobility for each single gene indicates that high recombination rate does not
235 have impact on genes with critical adaptive functions as well as GOF and primary metabolic portfolios they
236 built over long evolutionary time scale when genes or their alleles are constantly shuffled by the high-rate
237 recombination until reach assortive locations and fixed by nature selection in a manner of cut-and-try. Be-
238 sides niche adaptation, nature also selects for higher genetic compatibility among metabolic pathways,
239 genes, and even alleles, such as the CAVs in our case, because conflicts among these components may
240 undermine the competence of host strains in constant competitions with adjacent sister strains (39). Thus
241 GOF and primary metabolic portfolios and their related genes may be resistant to recombination that re-
242 sults in allele substitution(40) and location rearrangement(41) because phylogenies with reduced inte-
243 grated fitness may be eliminated by nature selections. Recent studies have shown genetic interaction and
244 compatibility may create fitness cost and constrain the dissemination and evolution of dispensable genes
245 (42, 43), which similarly direct the transfer of genes and alleles intra- and inter-clade to confer Vig and Slu
246 distinct ecological and clinical features. Thus recombination accelerates the optimization of the genome
247 content to streamlines physiological functions and metabolic portfolios of host strains. High-rate recombi-
248 nation is able to help adaptive genes to sweep the population, which benefit recipient strains with competi-
249 tive advantages and faster evolution.
250

251 In conclusion, our empirical data regarding to the separation of clades Vig and Slu in species *E.*
252 *coli* clarify the role of recombination in population diversification and evolution. The extensive intra-clade
253 recombination interrupts linkage between genes and leaves them independent under selections, but do
254 not disarranged the optimized GOF and metabolic scheme of host strains possibly due to critical selection
255 for streamlined physiological activities. Meanwhile, the extensive recombination within a clade coheres
256 strains into a quasi-sexual population, but its intensity declines sharply as phylogeny distance increases,
257 which builds barriers between clades and diverge the population.
258

259 **Methods and Materials**

260 **Strains and MLST typing.** A world-wide collection of 202 *E. coli* strains from vertebrate hosts (13) was
261 kindly provided by Professor Shulin Liu (Genomics Research Center, Harbin Medical University, Harbin,
262 China). A total of 149 successfully resuscitated strains were subjected to MLST sequencing and construc-
263 tion of a maximum-likelihood phylogeny using RaxML with model GTR and 1,000 bootstraps, together with
264 59 complete genomes of human-host deposited in NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/) as
265 of July 2013 and seven draft genomes published by a study of environmental strains (14) from
266 ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria_DRAFT/.

267 **Genome sequencing, annotation, and phylogeny inference.** The 45 selected representative strains
268 were sequenced by using Hiseq 2000 and a paired-end sequencing protocol (2 × 100 bp run) at depth of
269 150×. Long reads are also added for contiguity from PacBio RS II with one or two cells per strain. After
270 quality-filtration using the SMRT Analysis software suite (Pacific Biosciences Inc.), *de novo* assembly was
271 performed according to the Hierarchical Genome Assembly Process (HGAP) workflow, and consensus
272 sequences were corrected using Pilon (v1.22) with the clean reads generated from Illumina Hiseq 2000.
273 Genomes were annotated with GeneMark.hmm and BLAST against the COG database and KEGG
274 GENES (www.genome.jp/kegg/genes.html). Orthologs were identified by using PGAP (44), and the anno-
275 tated genomes show apparent consistency when compared to the genome framework generated from
276 GAAP (http://gaap.big.ac.cn), a synteny of core genes on genomic scale (45). Phylotypes of all strains
277 were identified *in silicon* according to the presence of three phylotype-specific genes or fragments as pre-
278 viously described (46).

279 **Measurement of growth rate, survival rate, and mobility.** Refer to SI.

280 **Genome content similarity, gene distribution, and homologue distance between the Vig and Slu**
281 **clades.** The Bray–Curtis dissimilarity index d was calculated for paired genomes as 1 – [2 * Sij/(Si + Sj)],
282 where Sij is the number of genes shared by strains i and j, and Si, Sj as that in strains i and j, respectively.
283 Two-sample Kolmogorov-Smirnov test was applied to select ortholog significantly enriched in Vig or Slu,
284 when p <0.01. Antibiotic-resistance and Shiga-like toxin genes were identified by BLAST against CBMAR
285 database (http://14.139.227.92/mkumar/lactamasedb) and the protein sequences of the toxin from *E. coli*
286 O157:H7 (lcl|AB015056.1_prot_BAA88123.1_1 for A-subunit and lcl|AB015056.1_prot_BAA88124.1_2 for
287 B-subunit). Pairwise distance of orthologs was calculated by using Protdist in the Phylip package.

Metabolism reconstruction. The metabolic profile of each strain was reconstructed *in silico* using the genome-scale models (GSMs) as described previously (17). Briefly, we first removed the pseudogenes in annotation and used ModelSEED (http://modelseed.org/) to build the genome-scale models based on flux balance analysis (18). Here we used the condition of "gram negative template" and "complete media" which contains 16279 compounds in KBase (ftp://ftp.kbase.us/assets/KBase_Reference_Data/Biochemistry). A compound was predicted to be consumed or produced when corresponding transport, metabolism or biosynthesis reactions were available in a strain.

Recombination inference. To infer genome-wide recombination, we utilized gmos (Genome MOsaic Structure) to make local alignments between paired genomes and identify identical fragments over 600bp (47) as candidate recombinant fragments. We further used the syntenic information of these candidates which parsimoniously retain those changed locations as authentic recombination fragments. Gene mobility was calculated as the percentage of being recombined in total genome pairs the gene was present. The recombination of a gene was also identified using the syntenic information as what for recombinant fragments.

Data Availability. The genome sequences we contribute are available on the NCBI GenBank (accession No. CP012760, CP012781, CP029328, CP029327, CP028732-CP028772) and Genome Sequence Archive of GSA database (http://gsa.big.ac.cn, Accession No. GWHAACT- GWHAAEJ). Raw data of sequencing are deposited in the GSA database (PacBio, Accession No. CRR028990-CRR029033; Hiseq, Accession No. CRR019978-CRR020022).

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316 **Author contribution:** J.Y. and Y.K. conceived the project and led the writing; L.Y., Y.C., R.F., and J. W.
317 compiled the data; and X.S., F.C., Z.H., Z.G., X.J., Q.L, Q.M., J.W., J.X., and S.H. analyzed the data. All
318 authors contributed to the writing and/or intellectual development of the manuscript.

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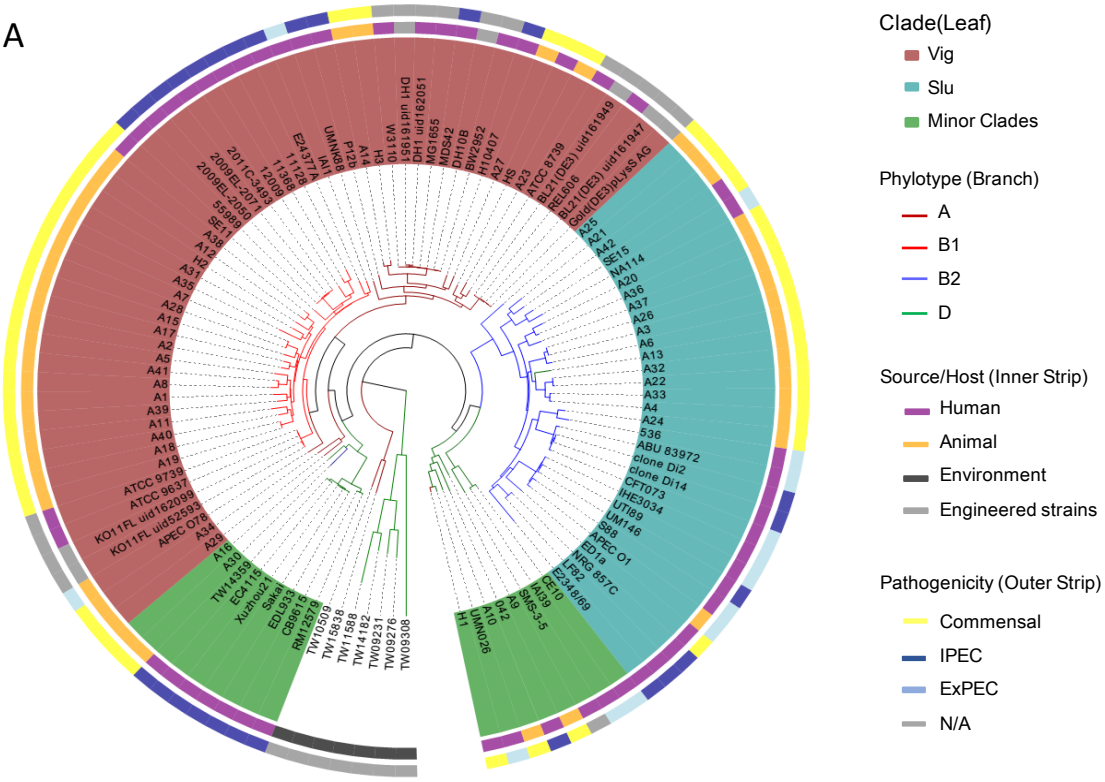
Figures and legends

A



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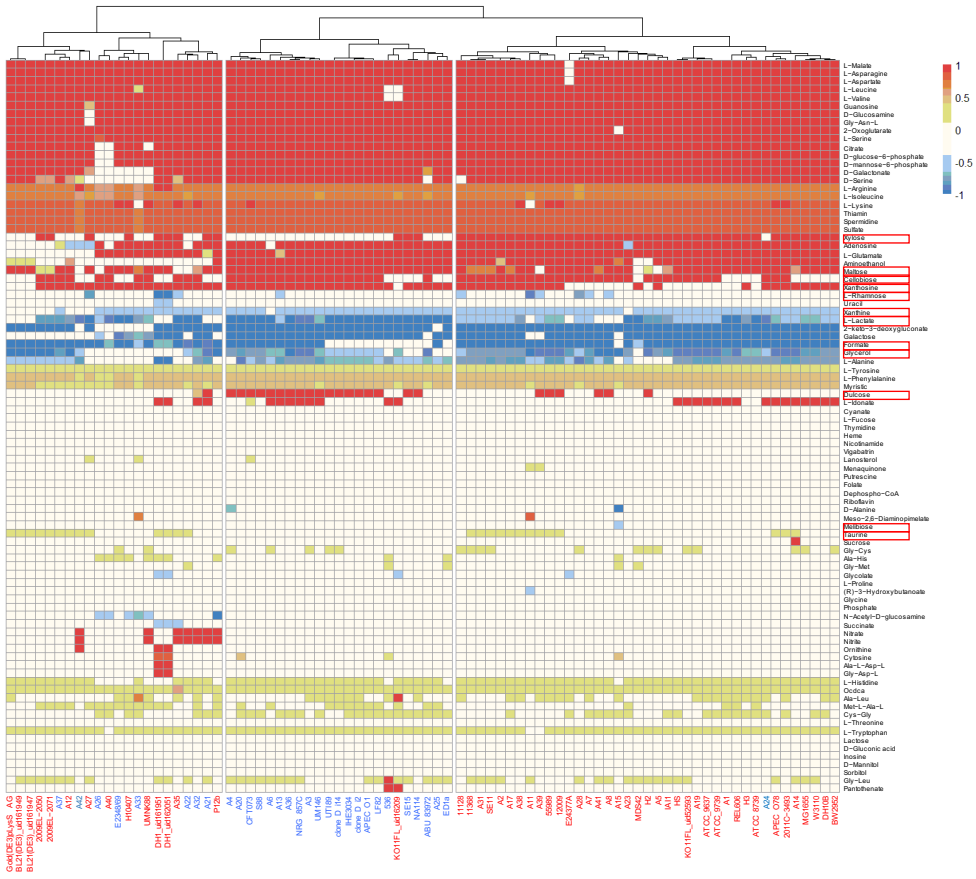
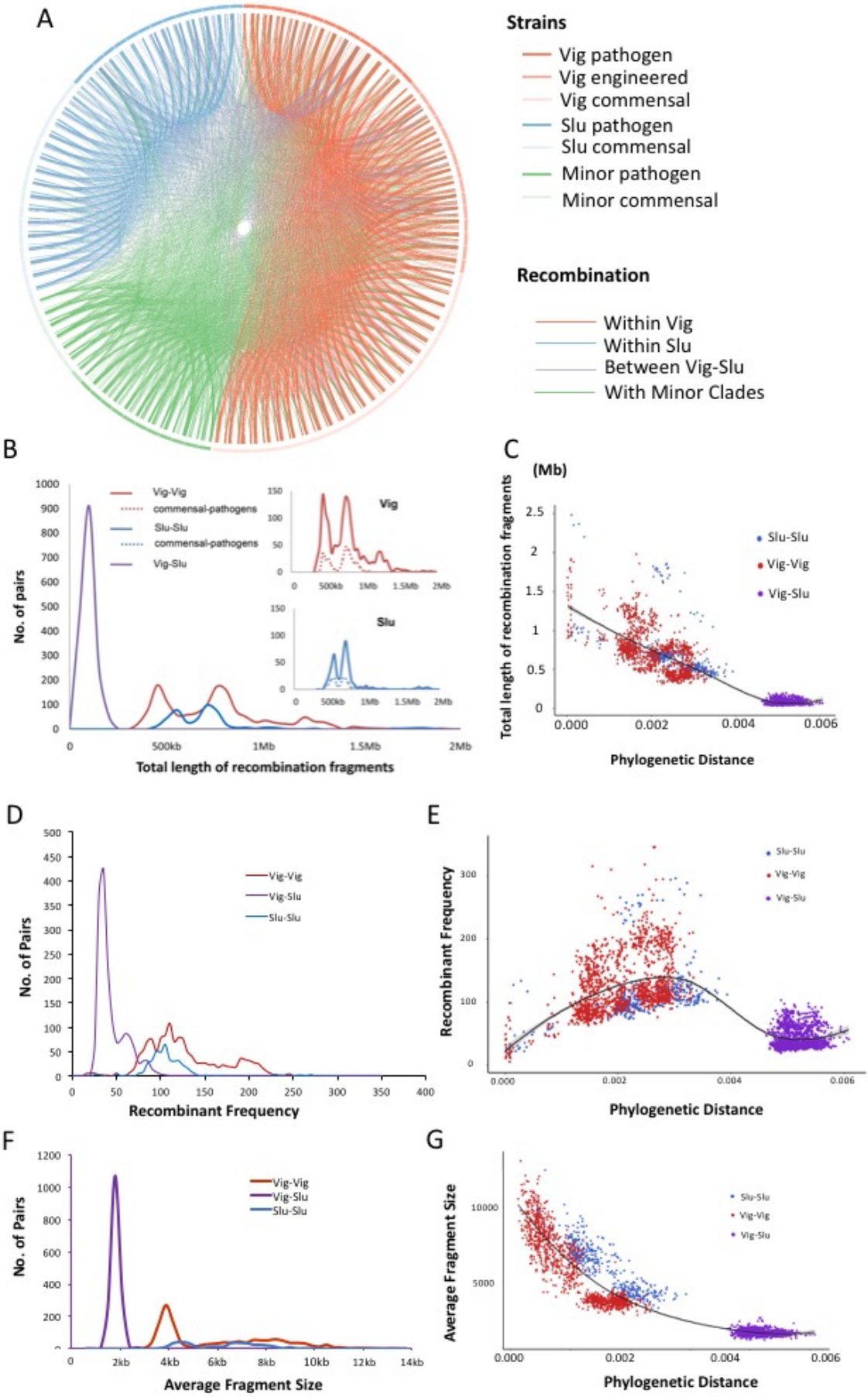


444

445 **Figure 1. The two major *E. coli* clades: Vig and Slu.**

446 (A) An ML phylogeny based on the core-genome derived polymorphisms that separate host-related
447 strains from the environmental strains (free-living in the tidal zone) and host-related strains into two
448 major clades and a few minor clades. Names of each strain are labelled. Clade (colored leaf), phylo-
449 type (colored branch), origin (colored inner stripe), and pathotype (colored outer stripe) are indicated
450 for each *E. coli* strain. (B) Prediction of metabolic compounds each strain uptakes and produces
451 based on genome-scale models (GSMs) and genome annotations for the two clades. The cluster dia-
452 gram represents 82 metabolic compounds with quantitative differences among strains, and those with
453 significant inter-clade differences ($p < 0.01$, Wilcoxon test) are highlighted in red boxes. Each column
454 represents a single strain with name labeled in red or blue color for the Vig or Slu, respectively. The
455 top clustering is based on the Euclidean distance between strains and Ward clustering algorithms.
456 Compound flux is normalized by its maximal value and colored in red for consuming and blue for pro-
457 ducing (see the right scaling bar).

458


A
Strains
Vig pathogen
Vig engineered
Vig commensal
Slu pathogen
Slu commensal
Minor pathogen
Minor commensal
Recombination
Within Vig
Within Slu
Between Vig-Slu
With Minor Clades
B
Vig-Vig
commensal-pathogens
Slu-Slu
commensal-pathogens
Vig-Slu
Vig
Slu
No. of pairs
Total length of recombination fragments
C
(Mb)
Total length of recombination fragments
Slu-Slu
Vig-Vig
Vig-Slu
Phylogenetic Distance
D
Vig-Vig
Vig-Slu
Slu-Slu
No. of Pairs
Recombinant Frequency
E
Slu-Slu
Vig-Vig
Vig-Slu
Recombinant Frequency
Phylogenetic Distance
F
Vig-Vig
Vig-Slu
Slu-Slu
No. of Pairs
Average Fragment Size
G
Slu-Slu
Vig-Vig
Vig-Slu
Average Fragment Size
Phylogenetic Distance

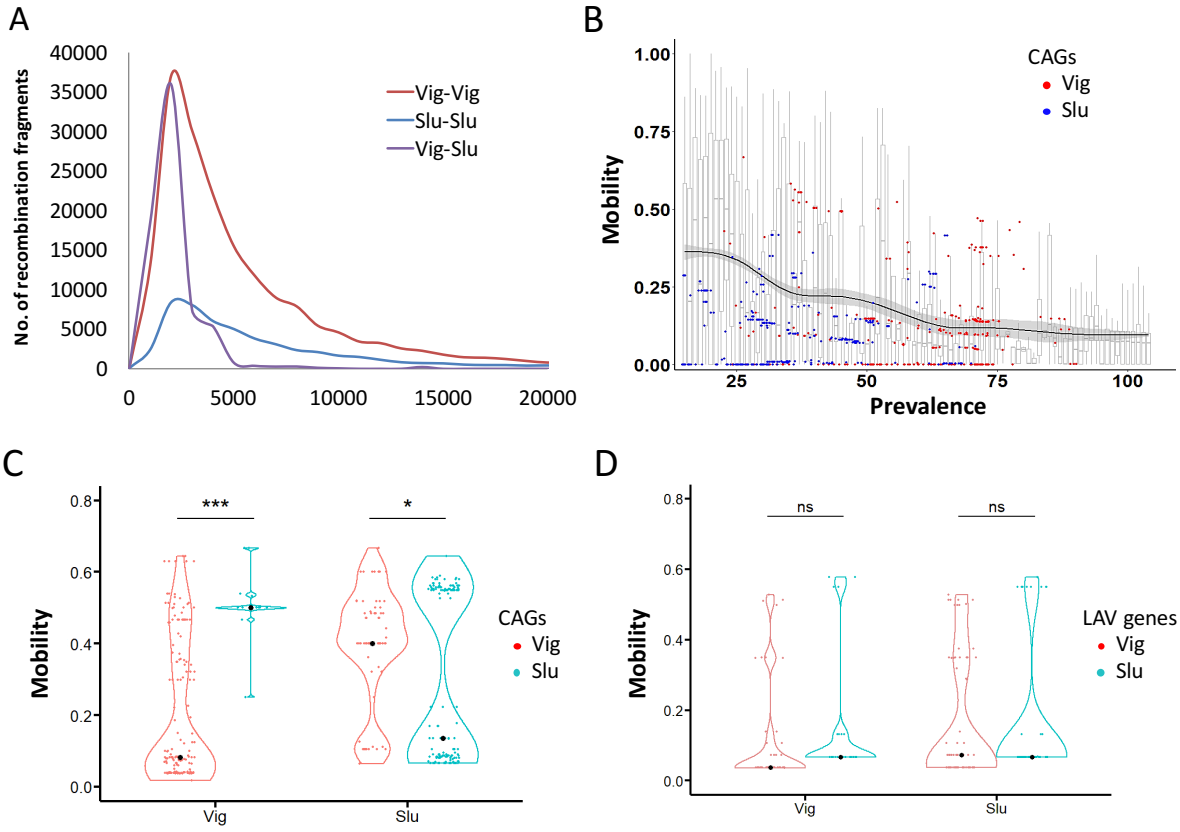
459

460 **Figure 2. Intra- and inter-clade Recombinations.**

461 (A) Pair-wise assessment of recombination within and across clades. Strains are circularly displayed
462 and the clades of Vig (red) and Slu (blue) are on both sides and the minor clade is placed (green) in
463 the middle. Pathogenic and commensal strains are indicated in dark and light colors. Each line be-
464 tween strains indicates recombination between the two strains with thickness proportional to the total
465 fragment length over 5kb. (B) Recombinant length distributions. Recombinants are grouped into intra-
466 clade (Vig–Vig, red; Slu–Slu, blue) and inter-clade (Vig–Slu, purple), and those between commen-
467 sal/pathogenic pairs (dotted line) are also shown in the insets. (C) Recombinant length is plotted as a
468 function of phylogenetic distance. (D) A plot of recombination frequency (number of recombinant frag-
469 ment). (E) Recombination frequency as a function of phylogenetic distance. (F) Average recombinant
470 length distribution. (G) Average recombinant length as a function of phylogenetic distance.

471

472



473

474 **Figure 3. Gene mobility.**

475 (A) Recombinant size distributions. Recombinants of Vig intra-clade (Vig–Vig), Slu intra-clade (Slu–
476 Slu), and inter-clade (Vig–Slu) are plotted as a function of size (bp) in red, blue, and purple, respec-
477 tively. (B) Gene mobility (migration flexibility among genomes within and across clades) as a function
478 of prevalence (the number of genomes that contain the gene). The regression curve (solid line) is
479 based on generalized additive model with the shadow representing 95% confidence interval. CAGs
480 (clade-associated genes) of Vig and Slu are indicated with red and blue solid circles, respectively. (C)
481 Mobility of CAGs in Vig (red) and Slu (blue). (D) Mobility of CAV (clade-associated variations)-contain-
482 ing genes in Vig (red) and Slu (blue).

483

484