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# 1 Phylogenetic background and habitat drive the genetic 2 diversification of *Escherichia coli*

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19 **Keywords:** local adaptation, gene repertoire, mobile genetic elements, horizontal gene  
20 transfer, freshwater isolates

## 23 **Abstract**

24 *Escherichia coli* is a commensal of birds and mammals, including humans. It can act as an  
25 opportunistic pathogen and is also found in water and sediments. Since most population  
26 studies have focused on clinical isolates, we studied the phylogeny, genetic diversification,  
27 and habitat-association of 1,294 isolates representative of the phylogenetic diversity of more  
28 than 5,000, mostly non-clinical, isolates originating from humans, poultry, wild animals and  
29 water sampled from the Australian continent. These strains represent the species diversity  
30 and show large variations in gene repertoires within sequence types. Recent gene transfer is  
31 driven by mobile elements and determined by habitat sharing and by phylogroup  
32 membership, suggesting that gene flow reinforces the association of certain genetic  
33 backgrounds with specific habitats. The phylogroups with smallest genomes had the highest  
34 rates of gene repertoire diversification and fewer but more diverse mobile genetic elements,  
35 suggesting that smaller genomes are associated with higher, not lower, turnover of genetic  
36 information. Many of these small genomes were in freshwater isolates suggesting that some  
37 lineages are specifically adapted to this environment. Altogether, these data contribute to  
38 explain why epidemiological clones tend to emerge from specific phylogenetic groups in the  
39 presence of pervasive horizontal gene transfer across the species.

40

## 41 Introduction

42 The integration of epidemiology and genomics has greatly contributed to our understanding  
43 of the population genetics of epidemic clones of pathogenic bacteria. However, the forces  
44 driving the emergence of these lineages in species where most clades are dominated by  
45 commensal or environmental strains remain unclear. *Escherichia coli* is a commensal of the  
46 gut microbiota of mammals and birds (primary habitat)<sup>1-3</sup>, and has been found in host-  
47 independent secondary habitats including soil, sediments, and water<sup>4-7</sup>. Yet, some *E. coli*  
48 strains produce virulence factors endowing them with the ability to cause a broad range of  
49 intestinal or extra-intestinal diseases (pathotypes) in humans and domestic animals<sup>8-13</sup>. Many  
50 of these are becoming resistant to multiple antibiotics at a worrisome pace<sup>14,15</sup>.

51 Studies on *E. coli* were seminal in the development of bacterial population genetics<sup>16</sup>. They  
52 showed moderate levels of recombination in the species<sup>3,17-19</sup>, and a strong phylogenetic  
53 structure with eight main phylogroups, among which four (A, B1, B2 and D) represent the  
54 majority of the strains and four others (C, E, F and G) are rarer<sup>20-22</sup>. Strains differ in their  
55 phenotypic and genotypic characteristics within and across phylogroups<sup>2,3,23,24</sup>, and their  
56 isolation frequency depends on factors such as host species, diet, sex, age<sup>25-27</sup>, body mass<sup>28</sup>,  
57 but also climate<sup>29,30</sup>, and geographic location<sup>31</sup>. Strains of phylogroups A and B1 appear to  
58 be more generalists since they can be isolated from all vertebrates<sup>2</sup> and are often isolated  
59 from secondary habitats<sup>7,32-35</sup>. *E. coli* strains able to survive and persist in water  
60 environments usually belong to the B1 phylogroup<sup>7,33,34</sup>. In contrast, the extraintestinal  
61 pathogenic strains usually belong to phylogroups B2 and D<sup>36-38</sup>. Genome size also differs  
62 among phylogroups, with A and B1 strains having smaller genomes than B2 or D strains<sup>23</sup>.

63 The phylogenetic vicinity of geographically remote *E. coli* isolates, and the co-isolation of  
64 phylogenetically distant strains, supports the hypothesis that strains circulate rapidly<sup>39,40</sup>. The  
65 genome of the species is also remarkably plastic, since only about half of the average  
66 genome is present across most strains of the species (core or persistent genome) and the  
67 pan-genome vastly exceeds the size of the typical genome<sup>41-44</sup>. Interestingly, the rapid

68 circulation of strains and the high plasticity of their genomes have not erased the  
69 associations of certain clades with certain isolation sources. In consequence, such  
70 associations might reflect local adaptation<sup>16,45</sup>, which would suggest frequent genetic  
71 interactions between the novel adaptive changes and the strains' genomic background.  
72 Understanding how the evolution of gene repertoires is shaped by population structure and  
73 habitats requires large-scale comparative genomics of samples with diverse sources of  
74 isolation representative of natural populations of *E. coli*. Most of the efforts of genome  
75 sequencing have been devoted to study pathogenic lineages and very few genomic data are  
76 available for commensal strains, especially in wild animals, and environmental strains. Here,  
77 we analysed the genomes of a large collection of *E. coli* strains collected across many  
78 human, domestic and wild animal and environmental sources in different geographic  
79 locations from the Australian continent. This collection is dominated by non-clinical isolates,  
80 corresponding to the main habitats of the species. We sought to understand the dynamics of  
81 the evolution of gene repertoires and how it was driven by mobile genetic elements. The  
82 analysis of the isolation sources in the light of phylogenetic structure and genome variation  
83 suggests that adaptation varies with the habitat and the phylogenomic background. This  
84 contributes to explain why known epidemiological clones of the species emerge from specific  
85 phylogenetic groups, even though virulence strongly depends on the acquisition of virulence  
86 factors by horizontal gene transfer.

## 87 Results

### 88 Very rapid initial divergence of gene repertoires becomes linear with time

89 We sequenced and annotated the genomes of 1,294 *E. coli sensu stricto* strains selected  
90 from more than 3,300 non-human vertebrate hosts, 1,000 humans and 800 environmental  
91 samples between 1993 and 2015, chosen to represent the phylogenetic diversity of the  
92 species (Materials and Methods, Fig. 1a, Supplementary Notes). All samples were collected  
93 by a single team, spanning a 20 year-period, from different regions in a single isolated  
94 continent (Australia). The origin of each strain was accurately characterized and the  
95 genomes were uniformly annotated and analyzed using the same bioinformatics processes.  
96 The strains were isolated from humans, domesticated and wild animals, representing the  
97 primary habitat of *E. coli*, and from freshwater, representing its secondary habitat<sup>3</sup>. Less than  
98 22% of the samples were recovered from clinical situations. A series of controls confirmed  
99 that the sequences were of high quality and contained the known essential genes  
100 (Supplementary Notes). The genomes varied widely in size from 4.2 to 6.0 Mb (average 5  
101 Mb), but had similar densities of protein-coding sequences (~87%) and GC content (50.6%,  
102 Supplementary Fig. 1 and Supplementary Table 1).

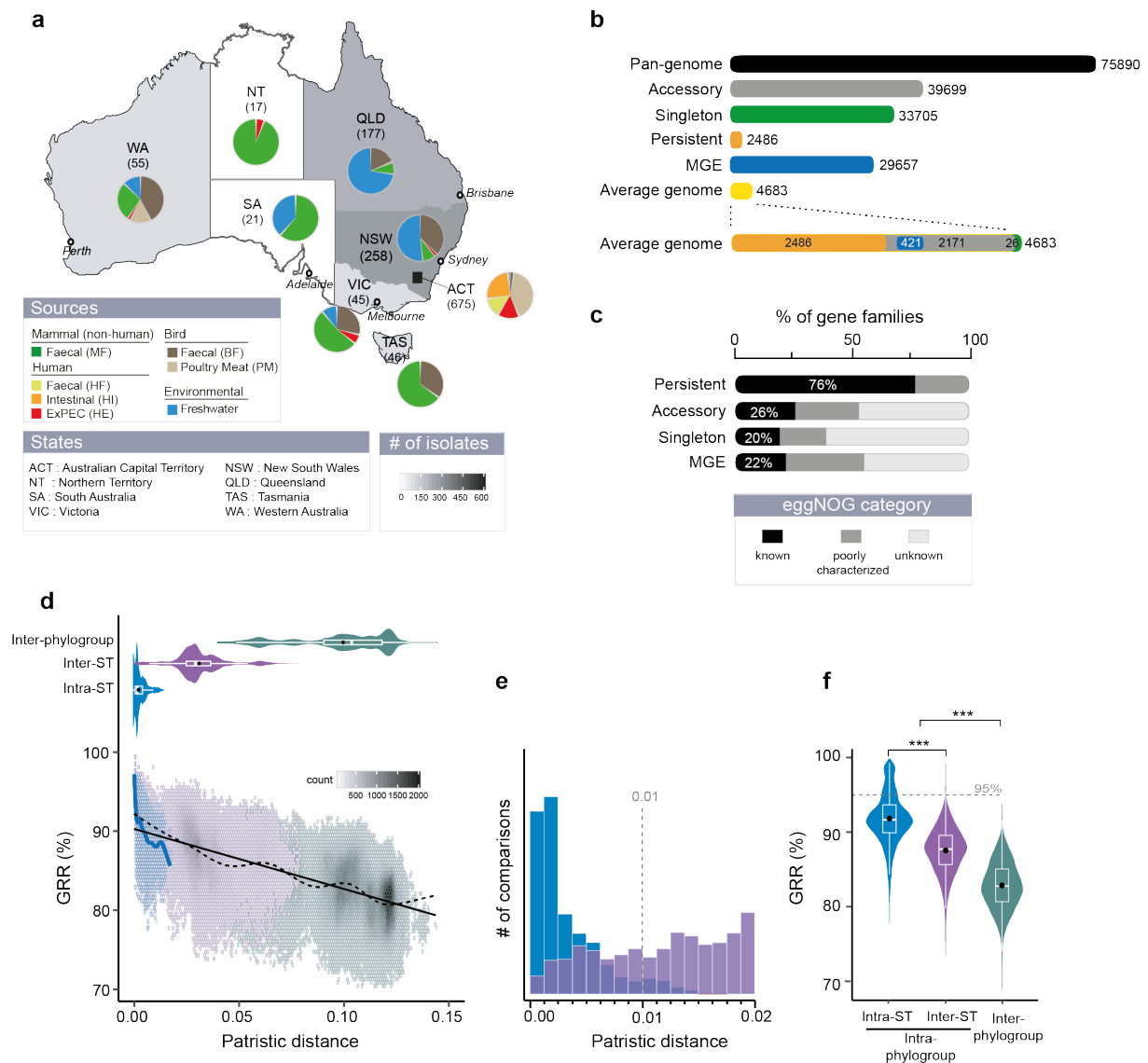
103  
104 The pan-genome contained 75,890 gene families that were classified as *persistent* (3%,  
105 gene families present in  $\geq 99\%$  of the genomes), *singletons* (44%, present in a single  
106 genome), or *accessory* (the remaining) (Fig. 1b, Supplementary Fig. 2). The persistent gene  
107 families are a tiny fraction of the pan-genome, but account for half of the average genome.  
108 They were used to build a robust phylogeny of the species, which was rooted using genomes  
109 from other species in the genus (Supplementary Fig. 3). In contrast, singletons are almost  
110 half of the gene families of the pan-genome, but less than 1% of the average genome. As a  
111 consequence, the pan-genome is open, as measured by the fit to a Heaps' law model<sup>46</sup>, and  
112 increases on average by ~26 protein coding genes with the inclusion of a new genome

113 (Supplementary Fig. 2). Singletons are smaller than the other genes and tend to be located  
 114 at the edge of contigs (44%). Hence, some of these singletons may result from sequencing  
 115 and assembly artifacts (Supplementary Notes and Supplementary Fig. 4). When all the  
 116 singletons were excluded, the pan-genome still remained open (Supplementary Fig. 2). Most  
 117 singletons (80%) and accessory (74%) gene families, but also a surprisingly high number of  
 118 persistent gene families (24%), lacked a clear functional assignment as given by the  
 119 EggNOG database<sup>47</sup> (Fig. 1c). Hence, we are still ignorant of the function, or even the  
 120 existence, of many genes of the species.

121

122 **Fig. 1: The genetic diversity of Australian *E. coli*.**

123



124

125 **(a)** Distribution of isolates per region and per source. **(b)** The pan-genome is composed of 75,890  
126 gene families, of which 33,705 are singletons (in green, present in a single genome), 2,486 persistent  
127 (in gold, present in at least 99% of genomes), the remaining being accessory (in grey). 29,657 gene  
128 families (39% of the pan-genome) were related to mobile genetic elements (MGE). **(c)** Percentage of  
129 the different EggNOG categories (see insert) in the persistent, accessory and singleton gene families  
130 and among genes associated to MGE. **(d)** [Top] Violin plots of the patristic distance computed  
131 between pairs of genomes. [Bottom] Association between GRR (Gene Repertoire Relatedness) and  
132 the patristic distance across pairs of genomes. Due to the large number of comparisons (points), we  
133 divided the plot area in regular hexagons. Color intensity is proportional to the number of cases (count)  
134 in each hexagon. The linear fit (black solid line, linear model (lm)) was computed for the entire dataset  
135 (1,294 genomes,  $Y=90.2-75.7*X$ ,  $R^2=0.49$ ,  $P<10^{-4}$ ). The spline fit (generalized additive model (gam))  
136 was computed for the whole (in black dashed line) or the intra-ST (in blue solid line) comparisons.  
137 There was a significant negative correlation between GRR and the patristic distance (Spearman's rho  
138 = -0.67,  $P<10^{-4}$ ). **(e)** Histograms of the number of intra-ST (in blue) and inter-ST (in purple)  
139 comparisons at short evolutionary scales. **(f)** Violin plots of the intra-ST, inter-ST and inter-phylogroup  
140 GRR (%). (d-e-f) All the distributions were significantly different (Wilcoxon test,  $P<10^{-4}$ ), the same color  
141 code was used and described in (d).

142 Traditional epidemiological studies of *E. coli* focused on multilocus sequence types (ST)  
143 and/or the O- and H-serotypes (often the O:H combination). These epidemiological units  
144 regroup strains in terms of sequence similarity in a few persistent genes (ST) or in key traits  
145 related to the cell envelope (the LPS structure and the flagellum). However, it is unclear if  
146 these types systematically regroup strains with similar gene repertoires. We identified 442  
147 distinct STs, of which 61% are represented by a single strain. A few STs are very abundant  
148 in our dataset: 20 include more than 10 genomes each and encompass 40% of the dataset.  
149 The intra-ST genetic distances are 10-times smaller than distances between other pairs of  
150 genomes (0.003 vs. 0.03, Fig. 1d). Yet, 6% of intra-ST comparisons have more than 0.01  
151 substitutions per position showing extensive genetic diversity at the genome level (Fig. 1e).  
152 Some O-groups are abundant, e.g., O8, O2 and O1 (each present in >50 genomes) but  
153 almost half of the groups occur in a single genome and 43% of the strains could not be  
154 assigned an O-group (even when the *wzm/wzt* and *wzx/wzy* genes were present). In contrast,  
155 most H-types were previously known (87%). We found 311 combinations of O:H serotypes  
156 among the 726 typeable genomes. Of these, 64% are present in only one genome, 17% are  
157 in multiple STs and 7% in multiple phylogroups (e.g. O8:H10). Conversely, half of the 95 STs  
158 with more than one genome have multiple O:H combinations, e.g. ST10 has 24. These  
159 results confirm that surface antigens and their combinations change quickly and are



160 homoplastic. They also show extensive variation of gene repertoires within STs. The gene  
161 repertoire relatedness (GRR) between genomes (see Methods) decreases very rapidly with  
162 phylogenetic distance for closely related strains, as revealed by spline fits (Fig. 1d). Similar  
163 results were observed when removing singletons, which only account for on average 0.5% of  
164 the genes in genomes, suggesting that this result is not due to annotation or sequencing  
165 errors (Supplementary Fig. 6). As a consequence, 85% of the intra-ST comparisons have a  
166 GRR lower than 95% (corresponding to ~235 gene differences per genome pair), and some  
167 as little as 77% (Fig. 1f). Hence, even genomes of the same ST can differ substantially in the  
168 sequence of other persistent genes and in the overall gene repertoires.

169  
170 To check if the dataset is representative of the species and can be used to assess its  
171 diversity, we compared it with the ECOR collection<sup>48</sup> and the complete genomes available in  
172 RefSeq (Materials). All datasets had similar nucleotide diversity (Supplementary Fig. 5a and  
173 Supplementary Table 1). Using rarefied datasets, to compare sets of same size, ours had the  
174 largest pan-genome, partly because of a larger number of singletons (Supplementary Fig.  
175 5b-d). Our dataset also had the highest  $\alpha$ -diversity for the three typing schemes (STs, O-, H-  
176 serotypes, Supplementary Table 1). Since the gene repertoire diversity of *E. coli* in Australia  
177 is at least as high as that of ECOR and RefSeq, we studied the variation in gene repertoires  
178 beyond the intra-ST level. After the rapid initial drop in GRR described above, the values of  
179 this variable decrease linearly with phylogenetic distances (Fig. 1d). The average values of  
180 GRR given by the regression vary between 90% for very close genomes and 80% for the  
181 most distant ones. The variance around the regression line is constant and a spline fit shows  
182 few deviations around the regression line. This is consistent with a model where initial  
183 divergence in gene repertoires is driven by rapid turnover of novel genes. After this initial  
184 process, divergence in gene repertoires increases linearly with patristic distance.

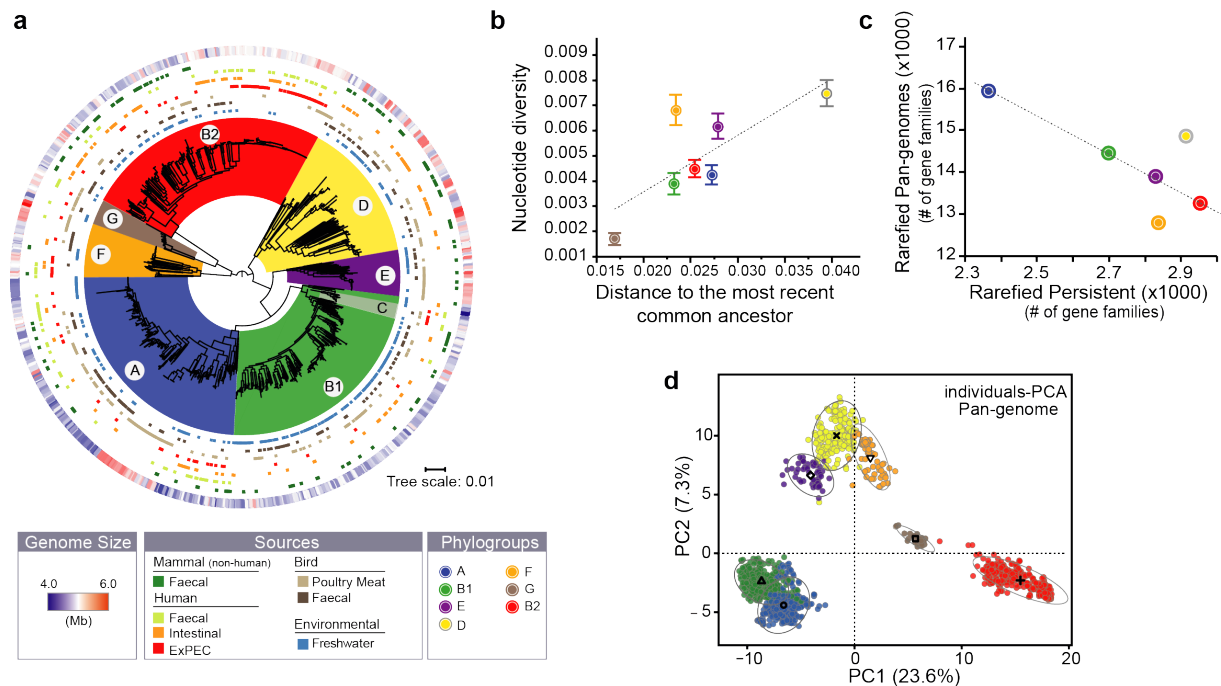
## 185 **Phylogroups vary in the rates of gene repertoire diversification**

186 We used the species phylogeny to study the associations between phylogroups and genetic  
187 diversity (Fig. 2a). The tree showed seven main phylogenetic groups very clearly separated  
188 by nodes with 100% bootstrap support. The 17 phylogroup C strains were all included within  
189 the B1 phylogroup and were thus grouped with the latter in this study. For the rest, the  
190 analysis showed a good correspondence between the assignment into the known  
191 phylogroups - A, B1, B2, D, E, F, and G – and the different clades of this tree. In line with the  
192 literature<sup>40</sup>, four major phylogroups were very abundant - A (24% of the dataset), B1 (24%),  
193 B2 (25%) and D (14%) – whereas the others were rarer. The nucleotide diversity of the  
194 phylogroups is very dependent on their phylogenetic structure, since some clades have more  
195 closely related clusters of strains than others (Supplementary Fig. 7). Nevertheless,  
196 nucleotide diversity, patristic distances, and Mash distances revealed similar trends: the  
197 phylogroup D exhibited the highest genetic diversity, followed by F, E, and then by the most  
198 abundant groups – A, B1 and B2 – which all have similar levels of diversity (Supplementary  
199 Fig. 7). The phylogroup G was the least diverse, but it is also poorly represented in our  
200 dataset (33 genomes from three STs). Overall, genetic diversity is proportional to the depth  
201 of the phylogroup, i.e. the average tip-to-MRCA distance, except for phylogroup F which is  
202 more diverse than expected (Fig. 2b). These results suggest that genetic diversity varies  
203 between phylogroups and that within phylogroups it is strongly affected by the time of  
204 divergence since the most recent common ancestor.

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**Fig. 2: The genetic and ecological structure of Australian *E. coli* population.**



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(a) Phylogenetic tree of *E. coli* rooted using the genomes of other *Escherichia* (not shown for clarity). From the inside to the outside: the 7 main phylogroups (arcs covering the tree), the source of each genome (seven rows), and the size of the genomes (outer row, see insert legend). (b) Association between the nucleotide diversity per site ( $P_i$ , average and s.e) within phylogroup and their distance to their most recent common ancestor (MRCA). In each phylogroup, we averaged the nucleotide diversity ( $\pi$ ) obtained for 112 core-genes, and the length branches (from tip-to-MRCA) of the species tree. (c) Association between the rarefied pan- and persistent-genomes in each phylogroup. We used 1,000 permutations (genomes orderings) of 50 randomly selected genomes (rarefied datasets) to compute the pan- and the persistent-genomes in each phylogroup (ignoring the G group), and then averaged the results. (d) Principal component analysis of the pan-genome (matrix of presence/absence of each gene family across genomes). Each dot corresponds to a genome in the two first principal components (PC). The ellipse (90%) and barycenter of each phylogroup are reported. The percentages in the axis labels correspond to the fraction of variation explained by the PC. Panels (b), (c), and (d) have the same color code as (a).

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The sets of genomes of each phylogroup have large and open pan-genomes (Supplementary Fig. 8 and Supplementary Table 2). The sizes of these pan-genomes differ widely across phylogroups and are partly correlated to the number of genomes in the phylogroup, explaining why the phylogroup G has the smallest pan-genome (Supplementary Fig. 8). To control for the effect of sample size, we computed pan-genomes from 1,000 random samples of 50 genomes for each phylogroup (ignoring the few strains of the G phylogroup, Fig. 2c and Supplementary Table 2). This revealed larger pan-genomes for phylogroups A, D, and B1 followed by E, B2 and F. Intriguingly, the larger the pan-genome of

232 a phylogroup, the smaller the fraction of its genes that are part of the persistent genome (Fig.  
233 2c). This suggests that differences of pan-genome sizes across phylogroups are caused by  
234 different rates of gene turnover in certain phylogroups. They affect all types of genes, even  
235 those at high frequency in the species.

236

237 To quantify the similarities in gene repertoires, we analyzed the GRR values between  
238 phylogroups. The smallest values were observed when comparing B2 strains with the rest  
239 (Supplementary Fig. 10). Accordingly, a principal component analysis of the  
240 presence/absence matrix of the pan-genome shows a first axis (accounting for 23.6% of the  
241 variance) clearly separating the B2 from the other phylogroups (Fig. 2d). This shows that  
242 gene repertoires of B2 strains are the most distinct from the other groups, even if B2 is not a  
243 basal clade in the species tree. Hence, phylogroups differ in terms of their gene repertoires  
244 and in their rates of genetic diversification.

### 245 **Mobile genetic elements drive rapid initial turnover of gene repertoires**

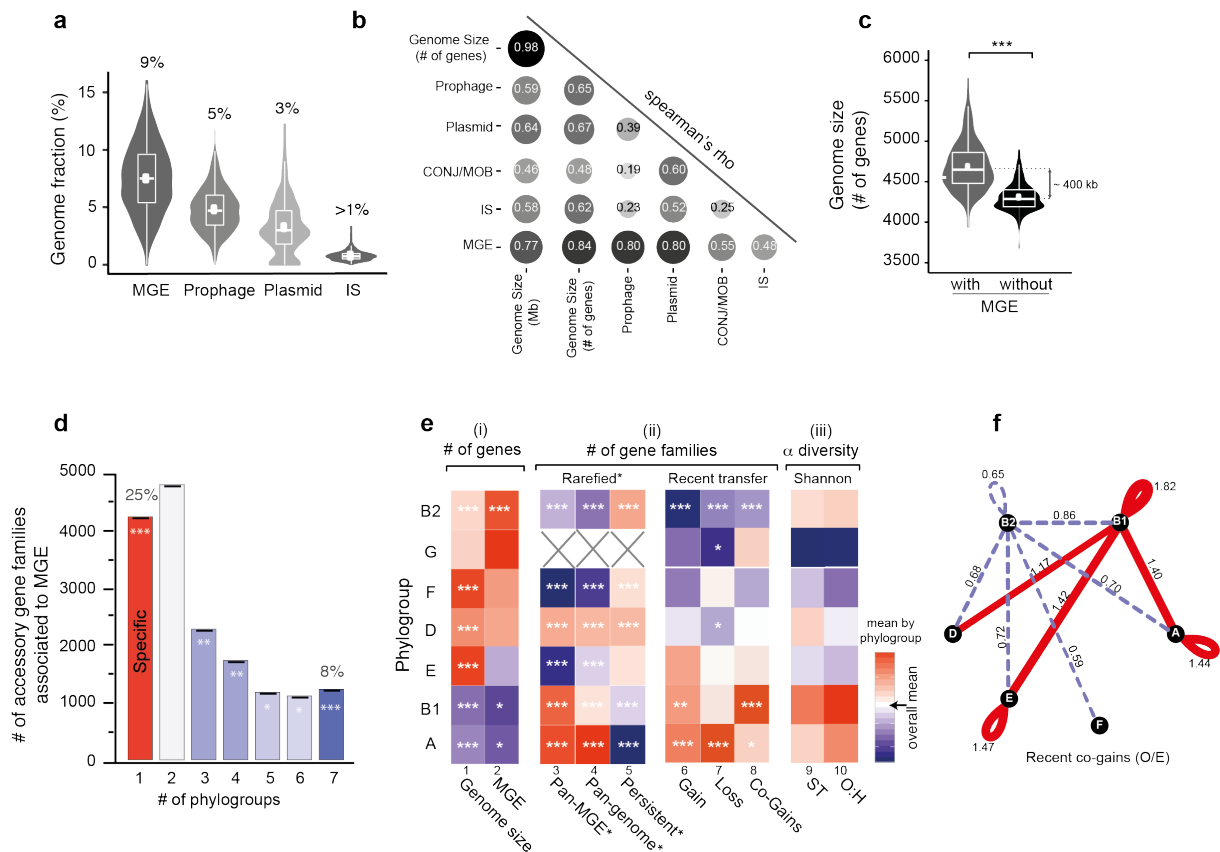
246 Different mechanisms can drive the rapid initial diversification of gene repertoires. Mobile  
247 genetic elements encoding the mechanisms for transmission between genomes (using  
248 virions or conjugation) or within genomes (insertion sequences, integron cassettes) are  
249 known to transfer at high rates and be rapidly lost<sup>49-51</sup>. We detected prophages using  
250 VirSorter<sup>52</sup>, plasmids using PlaScope<sup>53</sup>, and conjugative systems using ConjScan<sup>54</sup>  
251 (Supplementary Figs. 11-13). These analyses have the caveat that some mobile elements  
252 may be split in different contigs, resulting in missed and/or artificially split elements. This is  
253 probably more frequent in the case of plasmids, since they tend to have many repeated  
254 elements<sup>55</sup>. Only two genomes lacked identifiable prophages and only 9% lacked plasmid  
255 contigs. We identified 929 conjugative systems, with some genomes containing up to seven,  
256 most often of type MPF<sub>F</sub>, the type present in the F plasmid. On average, prophages  
257 accounted for 5% and plasmids for 3% of the genomes (Fig. 3a). Together they account for  
258 more than a third of the pan-genomes of each phylogroup. We also searched for elements

259 capable of mobilizing genes within genomes: Insertion Sequences, with ISfinder<sup>56</sup>, and  
 260 Integrons, with IntegronFinder<sup>57</sup>. Even if ISs are often lost during sequence assembly, some  
 261 genomes had up to 152 identifiable ISs representing ~1% of the genome (Fig. 3a and  
 262 Supplementary Fig. 13). A fourth of the ISs were in plasmids and very few were within  
 263 prophages. We found integron integrases in 14% of the genomes, usually in a single copy. It  
 264 is interesting to note that even if the frequency of each type of MGE varies across strains,  
 265 each of them is strongly correlated with the frequency of the other elements (Fig. 3b). Hence,  
 266 the typical *E. coli* genome has at least one transposable element, a prophage and a plasmid,  
 267 the key tools to move genes between and within genomes. When genomes are enriched in  
 268 one type of MGE, they tend to get simultaneously enriched in the remaining MGEs.

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270 **Fig. 3: Genetic diversification across phylogroups.**

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(a) Percentage of genes associated with MGEs per genome (sum in first graph). (b) Spearman's rank correlation matrix between the number of genes related to MGE (altogether or individually) and the genome size (in Mb and number of genes). Color intensity and the size of the circle are proportional to the correlation coefficients. All values are significantly positive ( $P < 10^{-4}$ ). (c) Differences in genome size when including or removing gene families associated to MGE (Wilcoxon test,  $P < 10^{-4}$ ). (d)

278 Number of accessory gene families associated to MGE present in one (i.e., phylogroup-specific) to  
279 seven phylogroups. The color code used corresponds to the Z-score obtained for the observed  
280 number (O) with respect to the random distribution (E) (see Methods) for each case with a color code  
281 ranging from blue (under-representation) to red (over-representation). The level of significance was  
282 reported: |Z-score| : \* ([1.96-2.58[), \*\* ([2.58-3.29[), \*\*\*([3.29). (e) Heatmap where a cell represents the  
283 deviation (the difference) of the phylogroup to the rest. All values were standardized by column. The  
284 color code ranging from blue (lower) to red (higher), with white (overall mean). The level of  
285 significance of each ANOM test was reported: \* (P<0.05), \*\* (P<0.01), \*\*\* (P<0.001). (f) Network of  
286 recent co-occurrence of gains (co-gains) of accessory genes within and between phylogroups. Nodes  
287 are phylogroups and edges the O/E ratio of the number of pairs of accessory genes (from the same  
288 gene family) acquired in the terminal branches of the tree. Only significant O/E values (and edges) are  
289 plotted (|Z-score|>1.96). Under-represented values are in dash blue and over-represented in red (see  
290 Methods).

291 What is the effect of these MGEs in the dynamics of *E. coli* genomes? First, the acquisition of  
292 MGEs affects the size of the genome. Those identified in this study account for ~8% of the  
293 genome size (Fig. 3c and Supplementary Fig. 14). Accordingly, the number of genes  
294 associated with MGEs was strongly correlated with genome size for every type of element  
295 (Fig. 3b). Second, MGEs increase the variability of genome sizes, since removing them  
296 decreases the coefficient of variation of the size of gene repertoires by 34% (expected  
297 increase of 4% under a Poisson model, Fig. 3c). Third, the increase in variance in genome  
298 size caused by MGEs is amplified by their short persistence times in the genome. No MGE-  
299 associated gene family is sufficiently frequent to be part of the persistent genome, and most  
300 (85%) are present in less than 1% of the genomes. For example, 41% of the IS gene families  
301 are singletons (Supplementary Fig. 14). Adaptive genes acquired through the action of  
302 MGEs may become fixed in populations, but the lack of fixation of recognizable MGEs  
303 suggests that the long-term cost of MGEs themselves is significant and/or their contribution  
304 to fitness is low (or temporary).

305

306 Is the distribution of MGEs associated with phylogroups leading to preferential paths of gene  
307 transfer? It has been suggested that homologous recombination is much rarer between than  
308 within phylogroups<sup>18</sup>. To test if this applies to the transfer of MGEs, we analyzed the  
309 distribution of the pan-genome gene families that are part of MGEs (excluding singletons, for  
310 the separate analysis of prophages and plasmids, see Supplementary Fig. 15). Even if these

311 genes are at low frequency in the pan-genome and are observed in a single phylogroup  
312 more often than expected by chance (Z-score>20, see Methods), 75% of the phage and  
313 plasmid gene families were found in more than one phylogroup and 8% were found in all  
314 phylogroups (usually at low frequency, Fig. 3d). Accordingly, the number of gene families  
315 present in two to six phylogroups is barely lower, even if significantly so, than expected by  
316 chance. These results suggest that there is frequent transfer of MGEs across the different  
317 phylogroups. To test this hypothesis more precisely, we used Count to infer gene gain and  
318 loss events in the phylogenetic tree of the species (see Methods). We found that half of the  
319 recent gene acquisitions, i.e., those that took place at the level of the terminal branches of  
320 the species tree, are in families of genes of MGEs. Conversely, the acquisitions at the  
321 terminal branches correspond to 40% of the MGE genes of the species. Hence, MGEs are  
322 key players in genome diversification at the micro-evolutionary scale. They are transferred  
323 across phylogroups and many of them, even if present in several strains, were acquired  
324 independently and have just arrived in their host genome.

325

326 One might expect more genetic diversity in phylogroups with more MGEs and larger  
327 genomes. In apparent agreement with this hypothesis, genomes from phylogroups A and B1  
328 are significantly smaller than the others (Fig. 3e, col 1, ANOM tests,  $P < 10^{-3}$ ) and have fewer  
329 MGE-associated genes (Fig. 3e, col 2, ANOM tests,  $P < 0.05$ ). However, these phylogroups  
330 also have the largest diversity of gene families associated to MGEs (Fig. 3e, col 3, in both  
331 the full and rarefied datasets, both ANOM tests,  $P < 10^{-3}$ ), i.e. they encode fewer but more  
332 diverse MGEs. Furthermore, the phylogroups A and B1, in spite of having among the most  
333 recent common ancestors of the phylogroups (Fig. 2b), have the largest pan-genomes, the  
334 smallest persistent genomes, and the largest diversity of STs, and serotypes (Fig. 3e, in both  
335 the full and rarefied datasets, cols 4,5,9,10, ANOM tests,  $P < 10^{-3}$ ). This intriguing pattern  
336 suggests that the smallest genomes have the highest turnover of genes, not the lower rates  
337 of transfer. To test this hypothesis, we took the quantification of gene gains and losses at the

338 terminal branches of the species tree and computed the number of these events per  
339 phylogroup. We found that phylogroups A and B1 have the highest number of gene gains  
340 and losses per terminal branch (Fig. 3e, cols 6-7). In parallel, we quantified the number of  
341 recently acquired (terminal branches) gene pairs (co-gains) from the same gene family within  
342 a phylogroup (Fig. 3e, col 8) and between phylogroups (see Methods, Fig. 3f). The results  
343 were represented as a graph where the edges represent significantly fewer (dashed lines) or  
344 higher (solid lines) number of co-gains than expected by chance. We found that phylogroup  
345 B1 has significantly more co-gains of genes with other phylogroups than expected, while the  
346 inverse was observed for phylogroup B2. We reached similar results when considering only  
347 the co-gains associated with MGEs (Supplementary Fig. 15). These results are consistent  
348 with the separation of the B2 phylogroup from the others in the PCA analysis (Fig. 2d). They  
349 show that such separation is due to lower rates of transfer in B2, which leads to fewer co-  
350 gains within the phylogroup and between this and the other phylogroups. In summary,  
351 phylogroups differ in terms of their genome size and in their rates of genetic diversification,  
352 the two traits being inversely correlated within the species.

### 353 **Not everything is abundant everywhere: the interplay between phylogroups** 354 **and sources**

355 Frequent horizontal transfer across phylogroups could result in adaptation being independent  
356 of the strain genetic background, if there is a lack of epistatic interactions. While we observed  
357 that all isolation sources have strains from all phylogroups (Fig. 4a), different phylogroups  
358 are typically over-represented depending on the source (Fig. 4b). These observations match  
359 previous studies<sup>3</sup>, and suggest strong associations between the phylogenetic structure of  
360 populations and the natural habitats of strains.

361 How much of the variability in gene repertoires is explained by the source of isolation of the  
362 strains? Genome sizes vary significantly across isolation sources. Strains isolated from  
363 poultry meat had the largest average genomes, followed by ExPEC strains. In contrast,  
364 strains from wild birds' feces and freshwater had the smallest genomes (Fig. 2a and Fig. 4c,

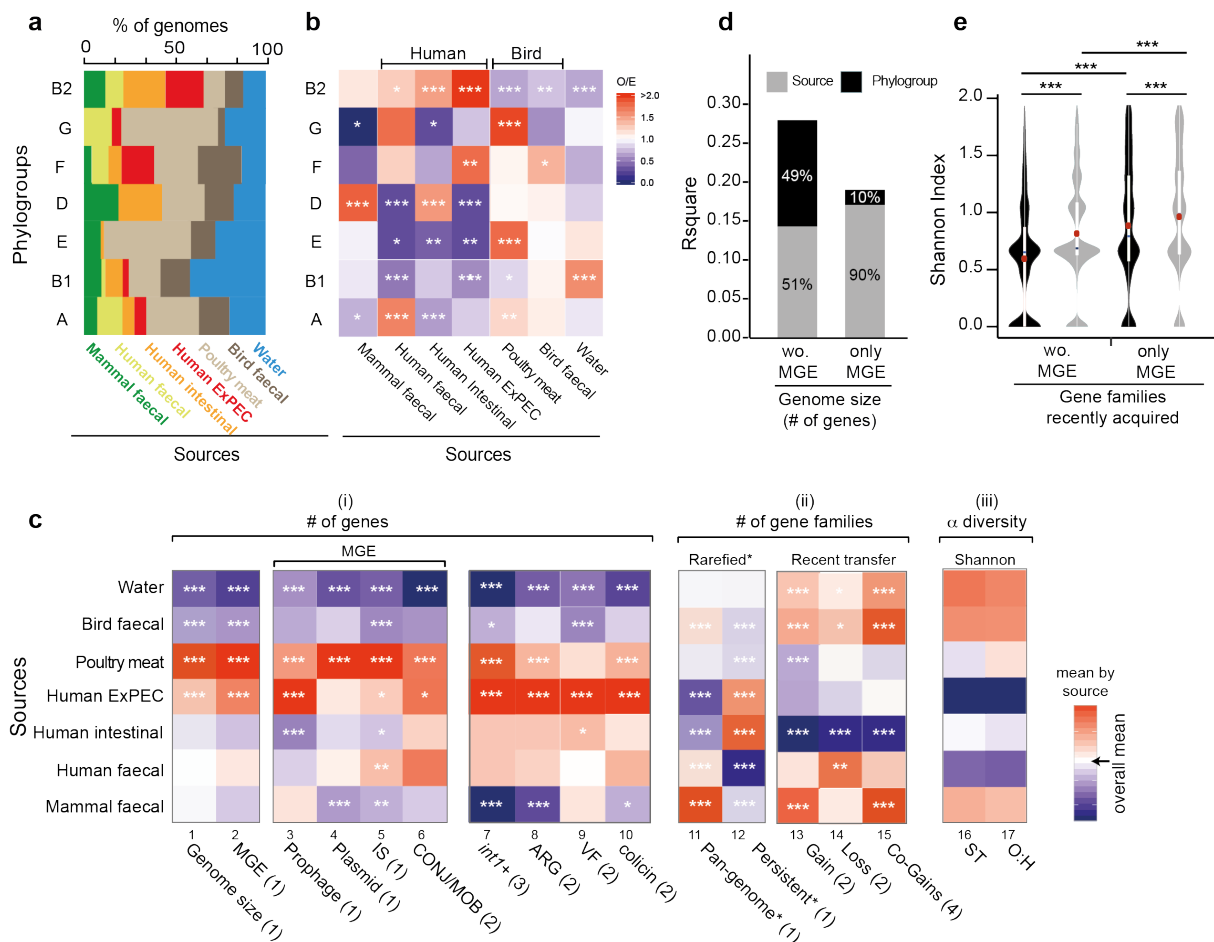


365 col 1, ANOM tests,  $P < 10^{-3}$ ). We showed above that genome size also varies across  
 366 phylogroups. To understand the relative role of the two variables, isolation source and  
 367 phylogroup, we made two complementary analyses. First, we compared the genome size of  
 368 strains from different sources within each phylogroup. Even if the statistical power was  
 369 sometimes low, this revealed trends similar to the ones observed across phylogroups  
 370 (Supplementary Fig. 17). Second, we used stepwise multiple regressions to assess the  
 371 effects of phylogroup and the strains' source on its genome size. Both variables contributed  
 372 significantly, and in almost equal parts, to the statistical model and together explained 36% of  
 373 the variance ( $R^2 = 0.36$ ;  $P < 10^{-4}$ , Supplementary Table 3). We found similar results after  
 374 removing MGE-associated genes (Fig. 4d and Supplementary Table 4). We conclude that  
 375 both isolation source and phylogroup are equally associated with genome size.

376

377 **Fig. 4: Genetic diversification across sources**

378



380 (a) Distributions of the sources in each phylogroup. (b) Association between phylogroups and sources. The ratio  
381 of the number of observed (O) genomes divided by the expected (E) number was reported for all comparisons  
382 with a color code ranging from blue (under-representation) to red (over-representation) (Fisher's exact tests  
383 performed on each 2\*2 contingency table). (c) Heatmap showing the associations between isolation sources and  
384 a number of traits. Each cell indicates the deviation (the difference) to the overall mean (in white). All values were  
385 standardized by column. By default, tests used standard ANOM (1). In presence of deviations from Gaussian  
386 distributions, we used non-parametric ANOM tests (2). We used ANOM for proportions (3). We represented the  
387 (O/E) ratio of the co-occurrence of gene pairs recently acquired (Co-gains) in each phylogroup with the same  
388 color code as in panel (b) (4). (d) Contribution of each variable (phylogroup and source) to the variance explained  
389 by the stepwise multiple regressions of genome size (for the component of MGEs or the remaining genome) on  
390 phylogroup and the isolation source. (e) Differences in diversity of gene families recently acquired across  
391 phylogroups (in black) and sources (in grey) for gene families associated to MGE or the remaining gene families  
392 (Wilcoxon tests, red dots (means)). In all panels : the level of significance of each test was reported: \*  
393 (P<0.05), \*\* (P<0.01), \*\*\* (P<0.001).

394

395 Adaptation to a habitat depends on HGT, which is driven by MGEs. Hence, we studied the  
396 distribution of MGEs in relation to isolation sources. There are fewer MGE-associated genes  
397 in strains isolated from freshwater and wild birds' feces, which have smaller genome sizes,  
398 and more in strains from ExPEC and poultry meat (Fig. 4c, col 2, ANOM tests,  $P<10^{-3}$ , and  
399 Supplementary Table 5). We observed similar trends within each phylogroup even if the  
400 statistical power was low (Supplementary Fig. 17). The analysis of the relative contribution of  
401 phylogroups and isolation sources to the number of MGE-associated genes showed that the  
402 source of the strain accounted for the vast majority of the explained variance (90%, full  
403 model:  $R^2=0.19$ ;  $P<10^{-4}$ , Fig. 4d and Supplementary Table 6). Accordingly, the number of  
404 MGE-associated gene families specific to a given source was higher than expected (Z-  
405 score >17, Supplementary Fig. 15), and nearly one third of these source-specific families  
406 were observed in multiple phylogroups. When we focused on the number of co-occurring  
407 recently acquired gene pairs (encoding for MGE or not), we found that they are more  
408 frequent within most of the isolation sources than expected by chance (Fig. 4c, col 15, see  
409 Methods). These results suggest that the contribution of MGEs to genome size is primarily  
410 driven by isolate source rather than phylogroup membership.

411

412 The previous result could arise from preferential co-gains of MGEs in an isolation source  
413 relative to a phylogroup. To test this hypothesis, we used the results from Count and built a  
414 matrix where for each gene family we indicate the acquisition or not of a gene in a terminal

415 branch of the phylogenetic tree. We then compared the clustering of these recent  
416 acquisitions by phylogroup and by isolation source using Shannon indexes (see Methods). If  
417 the hypothesis is correct, we expected higher clustering (lower diversity) across sources than  
418 across phylogroups. We observed slightly higher clustering across phylogroups than across  
419 sources, both for MGE-associated and for the other genes (Fig. 4e). We conclude that the  
420 contribution of MGEs to genome size depends largely on the isolation source but that this  
421 does not reflect systematic co-gains of MGEs in the same source.

422

423 It is tempting to speculate that the association between the number of MGE-associated  
424 genes and isolation sources reflects selection for the acquisition of locally adaptive functions  
425 transferred by these MGEs. To test this, we searched for the presence of antibiotic  
426 resistance genes (ARGs) in our dataset using the reference databases. Many of these ARGs  
427 were in integrons (~3 per integron), which is well documented<sup>58</sup>, and genomes carrying  
428 integrons had more ARGs than the others (Wilcoxon test,  $P < 10^{-4}$ , Supplementary Fig. 18).  
429 Expectedly, integrons and ARGs were more prevalent in ExPEC and in poultry meat isolates  
430 (Fig. 4c, cols 7-8) and Supplementary Table 5). Similar results were observed in the  
431 analyses at the level of each phylogroup (Supplementary Fig. 18). The clear association of  
432 integrons and ARGs with human (or domesticated animals) isolates of *E. coli* independently  
433 of the phylogroups' genetic background reinforces the idea that source-specific MGEs  
434 provide locally adaptive traits.

435

436 To complement the previous results, we searched for the presence of other factors known to  
437 be adaptive under specific conditions: virulence factors involved in antagonistic interactions  
438 with humans and colicins involved in intra-specific competition. Virulence factors (VFs) from  
439 VFDB are more prevalent in human strains with an excess in ExPEC isolates (ANOM test,  
440  $P < 10^{-3}$ ) and less frequent in strains isolated from freshwater and wild birds' feces (ANOM  
441 test,  $P < 10^{-3}$ , Fig. 4c, col 9). While VFs are more concentrated in phylogroups B2, D, E and F

442 (ANOM test,  $P < 10^{-2}$ ) as previously shown<sup>37</sup>, the trends regarding isolation sources are  
443 conserved within each phylogroup (Supplementary Fig. 19). In particular, within phylogroup  
444 B2, only human strains have a significantly higher average number of VFs (Supplementary  
445 Fig. 19) reinforcing previous results<sup>26</sup>. We also analyzed colicin gene clusters, which are  
446 agents of bacterial antagonistic competition and are often encoded in plasmids<sup>59</sup>. The  
447 average number of colicins identified using BAGEL3<sup>60</sup> (some of which are also included in  
448 VFDB) depends on the phylogroup of the strain, from an average of 2.8 genes in B2 strains  
449 to 0.4 in B1 strains. Interestingly, the water isolates have the fewest colicin genes,  
450 presumably because free diffusion of these proteins in water makes them inefficient tools of  
451 bacterial competition (Fig. 4c, col 10 and Supplementary Fig. 19). Thus, local adaptations  
452 resulting from the acquisition of novel genes by HGT, involving antagonistic interactions with  
453 other bacteria or with the host, are associated preferably with certain phylogroups. This may  
454 result from specific genetic interactions in the different genetic backgrounds.

455  
456 *E. coli* has usually been regarded as a contaminant from animal, mostly human, sources and  
457 used to test water quality. Yet, recent data suggests that some strains could inhabit aquatic  
458 environments<sup>61</sup>. Given the contrast between the primary and secondary habitats of *E. coli*,  
459 respectively guts of endotherms and aquatic environments, this would imply marked  
460 differences between the 285 freshwater strains and the others. Indeed, our results show that  
461 these strains are systematically different. They are over-represented in phylogroup B1 (43%),  
462 a phylogroup under-represented in all other sources of isolation (Figs. 2a,4b). On the other  
463 hand, they are under-represented in B2 (13%), a phylogroup over-represented in strains  
464 isolated from humans (this study) and other mammals<sup>2</sup>. The genome size of freshwater  
465 strains' is the smallest among all groups of isolates and across phylogroups (Fig. 4c, col 1,  
466 Supplementary Fig. 17). Importantly, these strains show average pan-genome sizes in the  
467 rarefied dataset, suggesting that adaptation is not exclusively due to genome reduction (Fig.  
468 4c, col 11). This is also supported by the high number of gains and losses observed (Fig. 4c,  
469 cols 13,14), although these genomes have the fewest MGEs and often lack plasmids (Fig. 4c,

470 cols 2-6). Consistent with adaptation to this habitat, they have the smallest number of  
471 antibiotic resistance genes, virulence factors, and bacteriocins (Fig. 4d, cols 7-10) and  
472 Supplementary Fig. 18,19). In contrast, these strains show the highest diversity of STs and  
473 O:H serotypes (Fig. 4c, cols 16,17, and Supplementary Table 5). The extreme genomic traits  
474 of isolates from water strongly suggest they are not the result of recent fecal contamination  
475 from other sources. Instead, they strongly suggest that these strains have changed to adapt  
476 to water environments. If so, this seems to have involved extensive horizontal gene transfer  
477 concomitant with streamlining, i.e. a high turnover of gene repertoires that resulted in  
478 genomes smaller than the average.

479

## 480 Discussion

481 Many of the recent advances in the understanding of *E. coli* evolution focused on clinical  
482 isolates and placed a lot of emphasis on virulence and antibiotic resistance in a few clinically  
483 important lineages<sup>62-67</sup>. Yet, most strains of the species are commensal. Hence, most of the  
484 evolution of the species takes place in biotic contexts not associated with pathogenesis.  
485 Furthermore, while a lot of attention has been placed on the rates of homologous  
486 recombination in the chromosome of the species, it is now clear that HGT drives the  
487 evolution of virulence<sup>12,42,68,69</sup> and antibiotic resistance<sup>70-72</sup> in pathogenic strains as well as  
488 that of many other traits in commensal strains<sup>12</sup>. For example, MGEs were recently shown to  
489 be more important than point mutations for the colonization of the mouse gut by *E. coli*  
490 commensals<sup>73</sup>. Here, we aimed at providing a global picture of the evolution of the *E. coli*  
491 genomes with an emphasis on the variation of gene repertoires in strains from a variety of  
492 sources (environmental and geographic) across a single continent. This allowed us to study  
493 the joint effect of population structure and habitat on the variation of gene repertoires. Our  
494 study focused on *E. coli* isolates from Australia, but its genetic diversity was higher or  
495 comparable to other worldwide genome datasets, and its population structure was consistent  
496 with previous works<sup>16,40,74</sup>. This indicates that what we have observed is likely to be  
497 representative of the species as a whole. It also confirms previous reports of the large  
498 genetic diversity of the species and of the planetary circulation of all major lineages<sup>39,45,75</sup>.  
499 Finally, the functional annotation of the pan-genome shows that in spite of over 375,000  
500 papers citing *E. coli* in PubMed in 2019, we are still far from having discovered the full  
501 genetic diversity of *E. coli* and from knowing the function of many of its most frequent gene  
502 families.

503

504 We started our study by quantifying gene repertoire diversification, which we found to follow  
505 a two-step dynamics. The very rapid initial diversification, where GRR quickly decreases to  
506 ~90%, implicates substantial heterogeneity in terms of gene repertoires for strains that are

507 from the same sequence type and are almost identical in the sequence of persistent genes.  
508 Some of this divergence may be due to genome sequencing or assembling artifacts  
509 producing singletons and thus inflating pan-genomes. Yet, we have annotated all genomes in  
510 the same way. We also confirmed key results by excluding singletons, and showed that  
511 singletons represent only ~0.5% of a typical genome and that many of them have homologs  
512 in the databases. The frequency of singletons is only weakly correlated with the number of  
513 contigs in draft assemblies, a further sign that they are not just caused by sequencing or  
514 assembly issues (Supplementary Notes). Furthermore, our analysis of ancestral genomes  
515 showed that a large fraction of well-known MGEs, including phages, ISs and plasmids, were  
516 acquired very recently (inferred acquisition at the terminal branches of the phylogenetic tree).  
517 Some of these are singletons, whereas others are present across many phylogroups. They  
518 contribute directly to the rapid divergence of gene repertoires between separating lineages.  
519 Previous population genetics models applied to other clades observed the existence of  
520 genes that have rapid turnovers in genes<sup>76,77</sup>. Our results show that frequent acquisition of  
521 MGEs drives rapid diversification of gene repertoires even between strains that are almost  
522 indistinguishable by classical typing schemes.

523

524 Following the abrupt initial loss of GRR between diverging lineages, we observed that the  
525 similarity of gene repertoires decreases linearly with time. Hence, it does not follow the  
526 negative exponential distribution that we proposed a decade ago<sup>42</sup>, which was based on a  
527 very small set of genomes that precluded the identification of the change of dynamics at  
528 small patristic distance. This change of dynamics resembles the accumulation of non-  
529 synonymous mutations in genes under weak purifying selection. Comparisons between  
530 closely related strains reflect almost neutral accumulation of recent events whereas  
531 differences between distant strains are driven by purifying selection with occasional fixation  
532 of adaptive events<sup>78,79</sup>. In the present context, this suggests that either many integrations of  
533 genetic material are slightly deleterious or that there is rapid deletion of neutral genes. The

534 first hypothesis is consistent with the fitness costs associated with the acquisition of many  
535 MGEs<sup>80-82</sup>, and with our observation that most MGEs present in a genome were very recently  
536 acquired. The second hypothesis is consistent with the previous works suggesting the  
537 existence of mechanistic biases towards gene deletion in bacteria<sup>83,84</sup>. Once most the recent  
538 transfer has been purged, by natural selection or gene deletion biases, GRR decreases  
539 linearly with divergence time and shows large variance around the regression line. The large  
540 variance indicates that some distantly related bacteria may have more similar gene  
541 repertoires than bacteria within the same sequence type. Importantly, the analysis does not  
542 suggest the existence of a point beyond which relatedness and gene flow change abruptly.  
543 Hence, these results do not suggest incipient sexual isolation within the species from the  
544 point of view of horizontal gene transfer. The analysis of gene flow associated with B2 strains  
545 should be placed in this context, it shows that this particular phylogroup has many MGEs and  
546 large genomes, but is recently exchanging less genetic material with strains from its own and  
547 from other phylogroups. This has placed it apart from the other phylogroups in terms of gene  
548 repertoires and in terms of preferential habitats.

549

550 The rapid evolution of gene repertoires by HGT is consistent with the observation that  
551 plasmids, prophages and ISs are almost ubiquitous among *E. coli*. These elements  
552 contribute to the genome size and especially to its variability across strains, which supports  
553 our previous results<sup>50,85</sup>. While most MGEs are quickly lost from lineages, or drive the lineage  
554 extinct, the large influx of such elements can bring adaptive accessory traits such as  
555 antibiotic resistance genes<sup>71</sup> and virulence factors<sup>86,87</sup>. They also pave the way for cooption  
556 processes<sup>88</sup>. The contribution of the MGE genes to genome size across the species is more  
557 strongly associated with the isolation source of the strains than with the phylogroup. However,  
558 the recent co-acquisition of MGEs by different strains is also associated with the phylogroup.  
559 This is consistent with a scenario where the abundance of MGEs in a genome is strongly  
560 dependent on the habitat, but their diversity also depends on the phylogroup. Since most



561 MGE genes arrived in the genome very recently, this suggests that habitat exerts a strong  
562 constrain on the flow of gene exchanges across *E. coli* strains, in line with the view that  
563 bacteria exchange more genes with those they coinhabit<sup>89,90</sup>.

564

565 The need of favorable genetic backgrounds for certain local adaptation processes could  
566 explain the observed over-representation of some phylogroups in certain isolation sources.  
567 Virulence factors and antibiotic resistance genes provide relevant examples. In our dataset,  
568 the plasmids encoding virulence factors are often conjugative and should be able to circulate  
569 widely, but the virulent clones often concentrate in only a few phylogroups. Selection for  
570 antibiotic resistance is expected to be higher in the virulent clones, because these are the  
571 most targeted in the clinic. Hence, they endure stronger selection to keep the ARGs arriving  
572 in MGEs. These causal links result in preferential associations of genetic backgrounds with  
573 virulence factors and ARGs, and therefore with the frequency of pathogens in a given  
574 phylogroup. How much of these trends are due to epistatic interactions between novel genes  
575 and the genetic background and how much is due to availability of specific genes by  
576 horizontal transfer in certain sources remains to be quantified. In conclusion, these results  
577 contribute to explain why epidemiological clones tend to emerge from specific phylogenetic  
578 groups even in the presence of massive horizontal gene transfer.

579

580 Genetic diversity, created by HGT, recombination, or mutation, affects a species' ability to  
581 adapt to novel ecological opportunities. The higher the diversity of gene repertoires in a  
582 population, the more likely that one of those genes will prove helpful in the face of  
583 environmental challenges such as antibiotics. We observed that the generalist phylogroups,  
584 such as A and B1, have broader pan-genomes than specialist phylogroups like B2. This was  
585 not expected based on their smaller genome sizes or the lower frequency of MGEs in their  
586 genomes. We propose that this reflects the high variability of the environments where they  
587 circulate and the consequent diversity of local adaptation processes. Phylogroup B2 strains,

588 by comparison, have developed very specific traits that may let them take advantage of  
589 some particular resources, e.g. they are better adapted to mammal gut environment<sup>2</sup>. This  
590 has resulted in large genomes that have diverged more from the other *E. coli*, as revealed by  
591 the PCA analysis, but that are overall more conserved (largest persistent-genome, smaller  
592 pan-genomes, fewer recent gene acquisitions). Altogether, these results suggest that the  
593 habitat and the phylogenetic structure jointly determine the size of genomes. The results also  
594 suggest the hypothesis that the large genomes of some phylogroups, like B2, are caused by  
595 a relative decrease in the rate of gene loss, not by an increase in the rate of gene gain.

596

597 The integration of information on gene repertoires and population structure in strains  
598 sampled from diverse sources can shed light on the origin of environmental strains. This is  
599 illustrated by the identification of genomic traits in freshwater *E. coli* isolates that are very  
600 different from the average traits of the species and that suggest adaptation of certain  
601 lineages to this environment. For bacteria, freshwater environments are much more nutrient  
602 poor than the guts of endotherms, and it's interesting to note that strains associated with this  
603 environment have more streamlined genomes. This may represent at the micro-evolutionary  
604 scale, an adaptation similar to that observed in other bacteria adapted to poor nutrient  
605 environments that have small genomes and few MGEs<sup>91,92</sup>. These results are also consistent  
606 with recent studies showing that *E. coli* B1 strains can persist longer in water than strains of  
607 the other phylogroups, and that B1 persistent strains in water often encode very few  
608 virulence factors and antibiotic resistance genes<sup>7,33,34</sup>. Interestingly these strains have been  
609 shown to be able to grow at low temperatures<sup>7</sup>. The prevalence of B1 isolates has been  
610 observed in other environmental samples, such as drinking water or plants<sup>93</sup>. The  
611 characteristics observed in freshwater isolates might be general to this environment, since  
612 they were observed in strains from the B1 and from other phylogroups (Supplementary Figs.  
613 16-18). If some *E. coli* lineages are indeed adapted to freshwater this radically changes the  
614 range of environments from where they can acquire novel genes and the selection pressures

615 that shape their subsequent fate. This finding also implies that environmental isolates are not  
616 necessarily the result of source-sink dynamics where *E. coli* strains evolve in relation to  
617 selection pressures linked to the host and environmental strains are just sinks where such  
618 strains find evolutionary dead-ends. Instead, the environment outside the host could have a  
619 significant impact on the evolution of *E. coli* subsequently colonizing human hosts.

620

## 621 **Materials and Methods**

622 **Strains:** We used different collections of *E. coli* strains recovered in Australia between 1993  
623 and 2015 (for a more detailed description, see Supplementary Note and Supplementary  
624 Dataset1). The subset of strains selected for whole genome sequencing includes : (1) *faecal*  
625 *strains* isolated from various birds (N=195 strains), non-human mammals (N=135), and  
626 humans living in Australia (N=93); (2) *clinical strains* isolated during intestinal biopsies of  
627 patients with inflammatory bowel disease (N=172), or corresponding to human ExPEC  
628 strains collected from urine or blood (N=112); (3) *poultry meat strains* isolated from chicken  
629 meat products from diverse supermarket chains and independent butcheries (N=283); (4)  
630 and *freshwater strains* isolated from diverse locations across Australia (N=285).

631  
632 **Sequencing:** Of the 1,304 isolates, 70 were sequenced at Broad institute using the Roche  
633 454 GS FLX system, 70 were sequenced by GenoScreen (Lille, France) using the  
634 HiSeq2000 platform and the rest were sequenced at the Australian Cancer Research  
635 Foundation (ACRF) Biomolecular Resource Facility (BRF) of the Australian National  
636 University using the Illumina MiSeq platform.

637  
638 **Assembling:** Paired-end read files were processed and assembled with CLC Genomics  
639 Workbench v.9.5.3 (Illumina) using their *de novo* assembly algorithm with default parameters.  
640 All genomes sequenced by the Broad institute were available into the NCBI Assembly  
641 ([www.ncbi.nlm.nih.gov/assembly/](http://www.ncbi.nlm.nih.gov/assembly/)) or SRA ([www.ncbi.nlm.nih.gov/sra/](http://www.ncbi.nlm.nih.gov/sra/)) databases. While, the  
642 rest of the assemblies was deposited into the European Nucleotide Archive (PRJEB34791).  
643 The accession number of each genome is reported in Supplementary Dataset1.

644  
645 **Datasets:** We used 4 datasets in this study. (1) The **Australian dataset** described above is  
646 the main dataset. (2) **RefSeq dataset:** We retrieved 370 *E. coli* complete genomes from  
647 GenBank Refseq (available in February 2018). (3) **ECOR dataset:** We retrieved 72 draft

648 genomes of the *E. coli* reference (ECOR) collection from DDBJ/ENA/GenBank<sup>48</sup>. Strains in  
649 this collection were isolated from diverse hosts and geographic locations and have been  
650 used for more than 30 years to represent the phylogenetic diversity of *E. coli* as they have  
651 been selected from over 2,600 natural isolates based on MLEE data<sup>17</sup>. (4) **Outgroup**  
652 **dataset**: We retrieved 65 other closely related *Escherichia* genomes from ENA/GenBank and  
653 sequenced 21 others on the Illumina MiSeq platform (assembled as described above). They  
654 belong to Clade I (N=14), Clade II (N=2), Clade III (N=8), Clade IV (N=2), Clade V (N=14), *E.*  
655 *fergusonii* (N=8) and *E. albertii* (N=38) species. Only five of them were complete, others were  
656 draft genomes. In this study, these genomes (called hereafter *outgroup* genomes) were only  
657 used to root the Australian *E. coli* species tree. The general genomic features and the  
658 sequencing status of these 1,832 genomes are reported in Supplementary Dataset1.

659  
660 **Data formatting**: In an attempt to overcome the bias from different annotations all genomes  
661 of the four datasets were annotated using Prokka v.1.11<sup>94</sup> which provided consistency across  
662 the entire datasets (with hmmer v.3.1b1, aragorn v.1.2.36, barrnap v.0.4.2, minced v.0.1.6,  
663 blast+ v.2.2.28, prodigal v.2.60, infernal v.1.1, ncbi\_toolbox v.20151127, and signalp v.4.0).  
664 We performed three quality controls on genomic sequences of Australian and outgroup  
665 datasets (see Supplementary Note). A total of 10 *E. coli* draft genomes and one genome  
666 from clade V failed at least one of these tests and were removed from further analysis,  
667 leading to a final dataset of 1,294 Australian *E. coli* genomes and 87 outgroup genomes. The  
668 main characteristics of each draft genome are reported in Supplementary Dataset1.

669  
670 ***E. coli* typing. Phylogroup**. The phylogroup of each *E. coli* genome (from ECOR, RefSeq,  
671 and Australian datasets) was determined using the *in silico* ClermonTyping method<sup>20</sup>.

672 **Multilocus sequence typing (MLST)**. Sequence type (ST) was identified by the MLST  
673 scheme of Achtman<sup>10</sup> using mlst v.2.16.1 (<https://github.com/tseemann/mlst>). We assigned  
674 STs for a large majority of genomes, i.e., for 99%, 96% and 97% of the ECOR, RefSeq and  
675 Australian genomes resp. **Serotype**. Serotype (O- and H-genotypes) was inferred with the

676 EcOH database<sup>95</sup> using ABRicate v.0.8.10 (<https://github.com/tseemann/abricate>). Currently  
677 there are 220 *E. coli* O-groups and 53 H-types described in this database. While 99% of  
678 Australian genomes had H-group assigned, only 57% had O-group assigned even if *wzm/wzt*  
679 and *wzx/wzy* genes are present. All these results are reported in Supplementary Dataset1.

680

681 **Nucleotide diversity.** The **nucleotide diversity** of the three datasets, *i.e.*, ECOR, RefSeq  
682 and Australian, was computed from the multiple alignments of 112 core gene families  
683 present in all *E. coli* genomes of these three datasets, (see below), using the `diversity.stats`  
684 function from the *PopGenome* v.2.6.1 R package<sup>96</sup>. We also used these 112 core gene  
685 families to assess the nucleotide diversity for each phylogroup of the Australian dataset.

686

687 **ST and O:H diversity.** The **Shannon index** was computed to assess the diversity of ST and  
688 O:H serotypes within each phylogroup and source. For this, we calculated their relative  
689 frequency in each group and then applied the function `skbio.diversity.alpha_diversity` from  
690 the *skbio.diversity* v.0.4.1 python package (<http://scikit-bio.org/docs/0.4.1/diversity.html>).

691

692 **Mash distances (M). Genome similarity.** Due to the high cost of computing ANI<sup>97</sup> via  
693 whole-genome alignment, we estimated genome similarity calculating the pairwise Mash  
694 distance (M) between all Australian genomes using Mash v.2.0<sup>98</sup>. Importantly, the correlation  
695 between the Mash distances (M) and ANI in the range of 90-100% has been shown to be  
696 very strong, with  $M \approx 1 - (ANI/100)^{98}$ . All the resulting Mash distances between *E. coli*  
697 genomes are well below 0.05, in agreement with the assumption that they all belong to the  
698 same species. The median is 0.027 and the maximal value is 0.04 (Supplementary Fig. 3).

699 **Australian *E. coli* reference genomes.** The Mash distance was strongly correlated to the  
700 patristic distance in our dataset (spearman's  $\rho=0.92$ ,  $P < 10^{-4}$ ). We used it to select 100  
701 Australian *E. coli* strains representative of the species' diversity (called hereafter *reference*  
702 genomes). Such *reference* genomes were used to root the Australian *E. coli* tree (to  
703 drastically reduce the computational time required to build the rooted tree). To select

704 representative genomes, we performed a hierarchical WPGMA clustering from the Mash  
705 distance matrix computed with all Australian *E. coli* genomes, and then we cut it off to have  
706 only 100 clusters. In each of these clusters, the genome with the smallest L90 was selected.  
707 This *reference* dataset contained all the phylogroups and was composed of: 15-A, 10-B1, 13-  
708 E, 39-D, 11-F, 10-B2 and 2-G genomes.

709

710 **Identification of pan-genomes:** Pan-genomes are the full complement of genes in the  
711 species (or dataset, or phylogroup) and were built by clustering homologous proteins into  
712 families. We determined the lists of putative homologs between pairs of genomes with  
713 MMseqs2 v.3.0<sup>99</sup> by keeping only hits with at least 80% identity and an alignment covering at  
714 least 80% of both proteins. Homologs proteins were then clustered by single-linkage<sup>100</sup>. We  
715 computed independently the pan-genome of each dataset, *i.e.*, ECOR, RefSeq, Australian  
716 and of the 87 outgroups with the 100 Australian *E. coli* reference genomes. Each pan-  
717 genome was then used to compute a matrix of presence-absence of gene families. Hence,  
718 gene copy number variations were not taken into account in this part of the study. The alpha  
719 exponent of Heap's Law was used to infer whether a pan-genome is open or closed<sup>46</sup>. Thus,  
720 if  $\alpha$  (alpha)  $\leq 1$ , the pan-genome is open. In contrast,  $\alpha$  (alpha)  $> 1$  represents a closed  
721 pan-genome. This coefficient was computed using the *heaps* function of the *micropan* v.1.2  
722 R package<sup>101</sup> with `n.perm = 1000`. Principal component decomposition of the Australian pan-  
723 genome, *i.e.*, the matrix of presence-absence of protein families was computed using the  
724 *prcomp* function from the *stats* v.3.5.0 R package.

725 The pan-genome of each phylogroup and source was taken from the pan-genome of the  
726 species. The pan-genome of the MGE (called **Pan-MGE**) was also taken from the species  
727 pan-genome and contained only genes encoding for MGEs.

728

729 **Rarefaction of pan-genomes:** The number of singletons was strongly correlated to the  
730 number of genomes analyzed in each phylogroup (Pearson's correlation = 0.97,  $P < 10^{-4}$ ),

731 indicating that the pan-genomes size depend on the number of genomes analyzed. Thus, to  
732 compare genetic diversity across datasets (e.g. phylogroups), we rarefied the genome  
733 datasets, *i.e.*, each pan-genome was constructed with the same number of genomes in each  
734 comparison. To do this, 1,000 subsets of  $X$  genomes ( $X$  depending on the analysis, specified  
735 in the results section) were randomly selected for comparison in each group, resulting to  
736 datasets called hereafter *rarefied* datasets (Supplementary Fig. 8).

737  
738 **Identification of persistent-genomes:** Gene families that are persistent were taken from  
739 the analysis of pan-genomes. A gene family was considered as persistent when it was  
740 present in a single copy in at least 99% of the genomes. We found 2,486 persistent gene  
741 families when considering the 1,294 Australian genomes, representing 52% of the average  
742 genome.

743  
744 **Identification of core-genome:** The core genome was taken from the analysis of the pan-  
745 genome. A gene family was considered as core if it is present in one single copy in all the  
746 genomes. To assess the nucleotide diversity, we built a core-genome with all the genomes of  
747 the ECOR, RefSeq, and Australian datasets. It was composed of 112 core gene families.  
748 Each gene family was aligned with mafft v.7.222 (using FFT-NS-2 method)<sup>102</sup>, and used to  
749 compute the average nucleotide diversity ( $\pi$ ) in each dataset and within each phylogroup  
750 (see above).

751  
752 **Functional assignment of the pan-genome:** Gene functional assignment was performed  
753 by searching for protein similarity with hmmsearch from HMMer suite v.3.1b2<sup>103,104</sup> on the  
754 bactNOG subset of the EggNOG v.4.5.1 database<sup>47</sup>. We have kept hits with an e-value lower  
755 than  $10^{-5}$ , a minimum alignment coverage of 50% of the protein profile, and when the majority  
756 (>50%) of non-supervised orthologous groups (NOGs) attributed to a given gene family  
757 pertained to the same functional group (category). The gene families that cannot be  
758 classified into any existing EggNOG clusters were grouped into the “unknown” category. Hits



759 corresponding to poorly characterized or unknown functional EggNOG clusters were grouped  
760 into the “poorly characterized” category.

761

762 **Phylogenetic analyses:** We built a rooted phylogeny of the species in two steps. **The**  
763 **phylogenetic species tree of Australian *E. coli*** was reconstructed from the concatenated  
764 alignments of the 2,486 persistent proteins of the 1,294 Australian *E. coli* strains. Each of  
765 these protein families was aligned with mafft v.7.222 (using FFT-NS-2 method)<sup>102</sup>. At this  
766 evolutionary distance the DNA sequences provide more phylogenetic signal than protein  
767 sequences. Hence, we back-translated the alignments to DNA, as is standard usage. We  
768 built phylogenies from persistent genomes to avoid the loss of signal associated with the  
769 small core genomes. When a genome lacked a member of a persistent gene family, or when  
770 it had more than one member, we added a stretch of gaps ('-') of same length as the other  
771 genes for it in the multiple back-translated alignments. Adding a few "-" has little impact on  
772 phylogeny reconstruction<sup>105</sup>. We have not removed recombination tracts from the multiple  
773 alignment because this has been shown to amplify errors in determining phylogenetic  
774 distances and it usually does not affect the topology of the tree<sup>106,107</sup>. If determination of the  
775 recombination was accurate in our >1,300 genomes dataset, this would have led to the  
776 exclusion of almost all the genes. The length of the resulting alignment for the species was  
777 2,298,168 bp. Each tree was computed with IQ-TREE multicore v.1.6.7<sup>108</sup> under the  
778 GTR+F+I+G4 model. This model gave the lowest Bayesian Information Criterion (BIC)  
779 among all models available (option -m TEST in IQ-TREE). We made 1,000 ultra-fast  
780 bootstraps to evaluate node support (options -bb 1000 -wbtl in IQ-TREE) and to assess the  
781 robustness of the topology of each tree<sup>109</sup>.

782 **The phylogenetic tree of *Escherichia* genus** was inferred from the persistent-genome  
783 obtained with the 87 outgroup genomes and the 100 *E. coli* reference genomes (see above)  
784 using the same procedure as the species tree. In this case, the persistent-genome is  
785 composed of 1,589 proteins families, and the resulting alignment of 1,469,523 bp. The genus  
786 phylogenetic tree was extremely well supported: all nodes had bootstrap support higher than  
32

787 95%. Its topology was consistent with a previous study<sup>110</sup> (Supplementary Fig. 3c). Then, we  
788 used it to precisely root the species tree (Supplementary Fig. 3d).

789 **The most recent common ancestor of each phylogroup:** We identified the node  
790 corresponding to the most recent common ancestor (MRCA) for each phylogroup from the  
791 rooted species tree using the *findMRCA* function from the *phytools* v.0.6.44 R package. Then,  
792 the subtree of each phylogroup was extracted using the *extract.clade* from the *ape* v.5.2 R  
793 package<sup>111</sup>. The distance to the MRCA was computed from the length of branches in each  
794 subtree. It corresponds to the average depth (distance from the MRCA) of all genomes (tips)  
795 within a phylogroup, and was inferred using the *depthTips* from the *phylobase* v.0.8.6 R  
796 package (<https://github.com/fmichonneau/phylobase>).

797  
798 **Evolutionary Distances:** For each pair of genomes, we computed a number of measures of  
799 similarity : 1) The **Patristic distance** was computed from the length of branches in the  
800 *Australian E. coli* species phylogenetic tree. The patristic distance is simply the sum of the  
801 lengths of the branches that link two genomes (tips) in the tree, and was inferred using the  
802 *cophenetic* function from the *ape* v.5.2 R package<sup>111</sup>. They were computed between all pairs  
803 of genomes, of the same ST (*intra-ST*), of different ST (*inter-ST*) within identical phylogroup,  
804 or of different phylogroups (*Inter-phylogroup*). As expected, we found that the *intra-*  
805 *phylogroup* (*both intra-ST and inter-ST*) patristic distances were significantly shorter than the  
806 *inter-phylogroup* (Wilcoxon test,  $P < 10^{-4}$ ). 2) **The Gene Repertoire Relatedness index (GRR)**  
807 between two genomes was defined as the number of common gene families (the intersection)  
808 divided by the number of genes in the smallest genome<sup>112</sup>. It is close to 100% if the gene  
809 repertoires are very similar (or one is a subset of the other) and lower otherwise. 3) **The**  
810 **Manhattan index** between two genomes is the number of different gene families. If two  
811 genomes have identical gene content, the corresponding Manhattan index is 0. 4) **The**  
812 **Jaccard index** between two genomes was defined as the number of common gene families  
813 (the intersection) divided by the number of gene families in both (the union). The Jaccard  
814 index between two genomes describes their degree of overlap with respect to gene family

815 content. If the Jaccard distance is 1, the two genomes contain identical protein families. If it is  
816 0 the two genomes are non-overlapping.

817 To characterize the genetic diversification of each phylogroup of the Australian dataset, we  
818 computed the three different standard indexes: the GRR, the Jaccard, and the Manhattan  
819 indexes. All these indexes were highly correlated ([Supplementary Fig. 9](#)). Thus, only  
820 analyses with GRR were reported and illustrated in the main text. Note that we always used  
821 the matrix of presence/absence of gene families to compute all these indexes, meaning that  
822 multiple occurrences were not considered. This downplays the impact of IS on pan-genome  
823 size and makes more conservative estimates of GRR divergence.

824

825 **Reconstruction of the evolution of gene repertoires:** We assessed the evolutionary  
826 dynamics of gene repertoires of the Australian genomes using Count (downloaded in  
827 January 2018)<sup>113</sup> with the Wagner parsimony method. Due to the size of our dataset it was  
828 not possible to do the analysis using birth-death models, but our previous analyses revealed  
829 very few differences between the two methods in smaller datasets<sup>114</sup>. Wagner parsimony  
830 penalizes the loss and gain of individual family members (with relative penalty of gain with  
831 respect to loss of 1, option  $g = 1$ ), and infers the history with the minimum penalty. Thus,  
832 from the pan-genome, *i.e.*, the matrix of presence-absence of gene families, and the rooted  
833 species tree, Count inferred the most parsimonious gain/loss scenario of each gene family  
834 along the tree. At each tree node, Count detailed information about individual families:  
835 presence/absence, and family events on the edge leading to the node. Hence, we have  
836 reconstructed the gene content of ancestral genome at each node. At each terminal branch,  
837 the expected total number of recent acquisitions (HGT) was computed by summing all family-  
838 specific gene gains obtained from the edge leading to the tip. Among them, we identified  
839 MGE associated genes that were recently acquired in each genome. We applied a similar  
840 strategy to identify recent losses.

841

842 **Distribution of accessory families across phylogroups (or sources):** We counted the  
843 number of MGE-associated gene families across phylogroups (Fig. 3d) or sources  
844 (Supplementary Fig. 15). We excluded the singletons from this analysis to avoid over-  
845 estimation of the number of families specific to one category. To test if some categories over-  
846 represented or under-represented these genes, we made 1,000 simulations. In each  
847 simulation, we shuffled the phylogroup (or source) assignment of the genomes while keeping  
848 the same number of taxa in each category (phylogroups or sources). Thus, the presence of a  
849 gene family in a genome and its frequency in the pan-genome remains the same, only the  
850 phylogroup (or the source) of genomes changes. The Z-score obtained for the observed  
851 number in the real data with respect to the random distribution (from 1,000 simulations) was  
852 reported for each case with a color code ranging from blue (under-representation,  $Z\text{-score} < -$   
853 1.96) to red (over-representation,  $Z\text{-score} > 1.96$ ).

854

855 **Recent co-occurrence of gains (co-gains) of gene families within phylogroups.**

856 We counted the number of recently acquired gene pairs (co-gains) from the same pan-  
857 genome gene family (see above) within and between phylogroups. Recently acquired genes  
858 were defined as those inferred as acquired in terminal branches using Count. To test if some  
859 phylogroups over-represented or under-represented these co-gains, we compared the  
860 observed number (O) within each phylogroup to the expectation (E) given by 1,000  
861 simulations. In each simulation, we shuffle the phylogroup assignment of the taxa (same  
862 approach as for the accessory gene families) and count the number of co-gains within and  
863 between phylogroups. For each phylogroup, we then divided the number observed in the real  
864 data (O) by the average number observed in the simulations (E), and computed the Z-score  
865 of the observed number (O) with respect to the random distribution (E). We considered an  
866 over(under)-representation significant when  $Z\text{-score} > 1.96$  ( $Z\text{-score} < -1.96$ ). Note that the O  
867 and E numbers had to be previously normalized (divided by the total number of gene pairs,  
868 i.e. the sum of pairs within and between phylogroups, in the real data, and in each

869 simulation, resp.). We applied the same approach (i) considering only gene pairs encoding  
870 for MGEs (similar result as in Fig. 3), (ii) for sources (instead of phylogroups, Fig. 4).

871

### 872 **Network of co-occurrence of gains (co-gains) of gene families across phylogroups.**

873 All co-gains (see above) were split into all possible combinations of phylogroup pairs (21  
874 combinations). To test if these co-gains are over- or under-represented between  
875 phylogroups, we compared the observed number (O) between each phylogroup to the  
876 expectation (E) given by 1,000 simulations with the same strategy as above. As before, we  
877 normalized the observed and expected numbers by the total number of co-gains in each  
878 simulation, calculated the (O/E) ratio, and the Z-score of each observed value in the real data  
879 with respect to the random distribution (E). The network was drawn using the *igraph* v.1.2.2  
880 R package (<https://igraph.org/r/>) with the circle layout option, where nodes are phylogroups,  
881 edges are (O/E) values for which the Z-score is significantly different from zero. The width of  
882 the edges is proportional to the (O/E) value and the color is blue for under- and red for over-  
883 representation (Fig. 3f). We applied the same approach considering only gene pairs  
884 encoding for MGEs (Supplementary Fig. 16).

885

886 **Gene family diversity:** We computed Shannon indexes to assess the diversity of each gene  
887 family recently acquired (terminal branches) across phylogroups and across sources (Fig.  
888 4e). If diversity is low, this means that acquisitions are clustered by phylogroup or source  
889 (depending on the analysis). For this, we calculated the relative frequency of each gene  
890 family recently acquired within each phylogroup (vs. each source). It is simply the number of  
891 genomes (within a phylogroup) with at least one acquisition divided by the total number of  
892 genomes in the phylogroup. We therefore obtained 2 vectors per gene family (one for  
893 phylogroups and one for sources) each containing 7 frequencies (for each phylogroup or  
894 each source) and then applied for each vector the function *diversity* from the *vegan* v.2.4.6 R  
895 package (<https://github.com/vegandevs/vegan>). If the index is 0, recent acquisitions of genes

896 of the family are limited to a single group (phylogroup or source). The higher the index, the  
897 more scattered the acquisitions of the family's genes are (across phylogroups or sources).

898

899 **Statistics:** All basic statistics were performed using R v 3.5.0, or *JMP*-13. (i) **Analysis of**  
900 **means:** We used **ANOM** to compare group means to the overall mean, when the data were  
901 approximately normally distributed. In cases where the data were clearly non-Gaussian and  
902 could not be transformed, we used the nonparametric version of the ANOM analysis, i.e.,  
903 **ANOM with Transformed Ranks**. It compares each group's mean transformed rank to the  
904 overall mean transformed rank. In both, we used the methods implemented in *JMP*-13. (ii)  
905 **Pairwise Wilcoxon Rank Sum Tests** were computed using the *pairwise.wilcox.test* function  
906 from the *stats* v.3.5.0 R package. We used the Bonferroni correction during multiple  
907 comparison testing. (iii) **Fisher's exact tests** were computed using the *fisher.test* function  
908 from *stats* v.3.5.0 R package. They were performed for testing the null of independence of  
909 rows (phylogroups) and columns (sources) in a 2x2 contingency table. (iv) **Correlation**  
910 **coefficients.** Pearson's and Spearman's rank correlation rho were computed using the *cor*  
911 function from *stats* v.3.5.0 R package. The correlation matrices were represented using the  
912 *corrplot* v.0.84 R package (<https://cran.r-project.org/web/packages/corrplot/index.html>). (v)  
913 **Smooth regression:** We used the generalized additive model (*gam*) smoothing method from  
914 the *mgcv* v.1.8.23 R package (<https://cran.r-project.org/web/packages/mgcv/index.html>). (vi)  
915 **Stepwise multiple regressions** were computed with *JMP*-13. This standard statistical  
916 method consists in a stepwise integration of the different variables in the regression by  
917 decreasing order of contribution to the explanation of the variance of the data<sup>115</sup>. We used  
918 the forward algorithm and the BIC criterion for model choice in the multiple stepwise  
919 regressions. The P-values associated with each variable were assessed using an F-test.

920

921 **Identification of Mobile Genetic Elements (MGEs): Prophages:** Prophages were  
922 predicted using VirSorter v.1.0.3<sup>52</sup> with the RefSeqABVir database in all genomes from  
923 Australian and RefSeq datasets, as a control. The least confident predictions, i.e., categories

924 3 and 6, were excluded from the analyses in both datasets. The prophage-associated  
925 regions in drafts are more numerous and shorter than in complete genomes (Supplementary  
926 Fig. 11). These results reveal that such regions are sometimes split in assemblies. In  
927 complete genomes, the cumulative size of the prophage-associated regions (X) is highly  
928 correlated with the number of prophages (Y) present in the genomes ( $Y=1.2923362 +$   
929  $1.6767 \cdot 10^{-5} X$ ,  $R^2=0.91$ ,  $P<10^{-4}$ , Supplementary Fig. 11). Hence, we used this linear equation  
930 to estimate the number of prophages in drafts using the cumulated size of prophage regions  
931 in the draft genomes. **Plasmids:** In the RefSeq dataset, all the extrachromosomal replicons  
932 were considered as plasmids. In the Australian dataset, plasmid sequences were identified  
933 using PlaScope v.1.3<sup>53</sup> with the database dedicated to *E. coli*. PlaScope provides a method  
934 for plasmid and chromosome classification of *E. coli* contigs. It has the specificity to select a  
935 unique assignment to each contig of a draft genome to plasmid, chromosome or unclassified.  
936 The number (~16, max: 124) and size (~9 kb, max: 166 kb) of contigs predicted as plasmid  
937 were highly variable (Supplementary Fig. 12) in the Australian dataset. Their size is much  
938 smaller than that of the average plasmid in complete genomes (~80 kb), reflecting the split of  
939 plasmids across different contigs because of the presence of repeated sequences, e.g. IS  
940 elements. Hence, we have not attempted to estimate the exact number of plasmids per  
941 genome and focus our analysis on the number of genes predicted to be in plasmid contigs.  
942 **MGEs (Plasmids + Prophages):** We found 11,864 gene families specifically related to  
943 plasmid elements, 14,188 to prophage elements, and 2,599 shared by both (9% of the MGEs  
944 gene families). In complete genomes, prophage and plasmids elements account for half of  
945 the pan-genome, of which 1 third were singletons. The large fraction of singletons from  
946 MGEs confirms that these elements are extremely diverse and evolved very rapidly, which  
947 underlines the difficulty of accurately detecting them and probably leads to their under-  
948 estimation in draft genomes. **Loci encoding conjugative or mobilizable elements** were  
949 detected with the CONJscan module of MacSyFinder<sup>116</sup>, using protein profiles and definitions  
950 following a previous work<sup>54,117</sup>. 87% of conjugative systems and 75% of putative mobilizable

951 elements were located on contigs predicted as plasmids by Plascope. **Integrans** were  
952 identified using IntegronFinder v.1.5 with the `-local_max` option<sup>57</sup>. 186 integron-integrase (*intl*)  
953 were detected with one quarter located at the edges of contigs. We only found one copy per  
954 genome. They were often located on very short contigs (20 proteins on average), and five  
955 make all the contigs. Most (86%) were located on contigs predicted as plasmid by Plascope,  
956 the remaining were on unclassified contigs. Except for the latter, *intl* genes were always  
957 located next to ARGs. **IS elements** were identified using ISfinder<sup>56</sup>. Only hits with an e-value  
958 lower than  $10^{-10}$ , a minimum alignment coverage of 50% and with at least 70% identity were  
959 selected, we extracted the IS name of the best hit. Therefore, we identified 47,592 genes  
960 encoded for IS elements, among them 43% were located at the edges of contigs  
961 (20,329/47,592). They represented 1,006 gene families (~1% of the pan-genome), of which  
962 41% were singletons. Only 13% were multigenic protein families (*i.e.*, with more than one  
963 member in at least one genome). Among them, 9 protein families were found in more than 10  
964 copies in at least one genome, *i.e.*, ISEc1 (10 copies), IS1397 (11), ISSoEn2 (11), IS621 (11),  
965 IS2 (15), IS629 (17), IS200C (17) IS1203 (18), and the most extreme case IS1F (107). Very  
966 large numbers of ISs, usually a sign of recent proliferation, was restricted to a small number  
967 of genomes (Supplementary Dataset1), but this may be an under-estimate caused by the  
968 loss of ISs in the assembling process. ISs were often fragmented, characterized by  
969 numerous singletons, and six times more frequently present at the edges of contigs than  
970 expected by chance. All the results are reported in Supplementary Dataset1.

971  
972 **Antibiotic resistance genes (ARG)** were detected using 2 curated databases of antibiotic  
973 resistance protein: Resfinder v.3.1<sup>118</sup> and ARG-ANNOT v.3<sup>119</sup>. Therefore, we used BlastP  
974 and selected the hits with an e-value lower than  $10^{-5}$ , with at least 90% of identity and a  
975 minimum alignment coverage of 50%. We found a strong positive correlation between the  
976 number of ARGs per genome using each database (pearson's  $r=0.97$ ,  $P<10^{-4}$ ). The main  
977 difference is the additional detection of three ARGs by ARG-ANNOT, *i.e.*, AmpC2, AmpH,



978 Mfd, which are persistent in Australian dataset and normally do not confer antibiotic  
979 resistance in *E. coli*. All the results are reported in Supplementary Dataset1.

980

981 **Virulence factors (VF)** were identified using VFDB (downloaded in February 2018, <sup>120</sup>). The  
982 two databases, *i.e.*, VFDB\_setA and VFDB\_setB were used independently. We used BlastP  
983 and selected the hits with an e-value lower than  $10^{-5}$ , at least 70% of identity and minimum  
984 alignment coverage of 50%. We found 1,332 (vs. 3481) gene families encoding virulence  
985 factors with the setA (vs. setB). In spite of these differences, we found qualitatively similar  
986 conclusion with the 2 sets because they are very correlated (pearson's  $r=0.97$ ,  $P<10^{-4}$ ). All  
987 the results are reported in Supplementary Dataset1.

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## 995 **Author Contributions**

996 D.G., B.V., S.B., and CL.B. collected, sequenced and assembled the Australian isolates. A.P.  
997 annotated the genomes and deposited them into ENA. M.T., E.D., D.G., and EPC.R.  
998 designed the research. M.T. managed the project and made most of the computational  
999 analyses. JAM.S. performed computational analyses. M.T and EPC.R analyzed the data.  
1000 M.T. and EPC.R. wrote the manuscript with input from other co-authors.

## 1001 **Competing Interests Statement**

1002 No conflict of interest.

## 1003 **Data accessibility**

1004 Data deposition: Genome assemblies have been deposited into the European Nucleotide  
1005 Archive (ENA) at EMBL-EBI under accession number PRJEB34791.

1006 This article contains supporting information online at url :

1007

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