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Why panmictic bacteria are rare

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Abstract

Background Bacteria typically have more structured populations than higher eukaryotes, but this difference is surprising given high recombination rates, enormous population sizes and effective geographical dispersal in many bacterial species.

Results We estimated the recombination scaled effective population size $N_e r$ in 21 bacterial species and find that it does not correlate with synonymous nucleotide diversity as would be expected under neutral models of evolution. Only two species have estimates substantially over 100, consistent with approximate panmixia, namely *Helicobacter pylori* and *Vibrio parahaemolyticus*. Both species are far from demographic equilibrium, with diversity predicted to increase more than 30 fold in *V. parahaemolyticus* if the current value of $N_e r$ were maintained, to values much higher than found in any species. We propose that panmixia is unstable in bacteria, and that persistent environmental species are likely to evolve barriers to genetic exchange, which act to prevent a continuous increase in diversity by enhancing genetic drift.

Conclusions Our results highlight the dynamic nature of bacterial population structures and imply that overall diversity levels found within a species are poor indicators of its size.

Keywords

Panmixia, bacterial population structure, effective population size, recombination, genetic diversity

Background

Bacteria are paragons of adaptability and make up more biomass than all organisms other than plants combined [1]. Many bacterial species have enormous population sizes, disperse effectively around the globe [2-5] and exhibit high rates of within-species homologous recombination [6]. Recombination progressively breaks down non-random associations between markers (linkage disequilibrium), so that in large populations, where pairs of individuals distantly related, linkage equilibrium is expected between all pairs of genomic sites. However, in most species for which data is available, there is substantial genome-wide linkage disequilibrium, indicating structuring of variation [7]. Here we

42 propose a resolution of this paradox, namely that large bacterial populations accumulate diversity

43 progressively until that diversity acts as an effective barrier to genetic exchange between lineages.

44

45 We examine the population structure of 21 bacterial species and find that only the Asian population of

46 *Vibrio parahaemolyticus* is close to genome-wide linkage equilibrium. We find that this population

47 has undergone a recent expansion. If the current population size was maintained over evolutionary

48 timescales, it would lead to a greater than 30 fold increase in diversity, to levels higher than found in

49 any well characterized bacterial species. We propose that other environmental species with large

50 census population sizes, for example *Vibrio cholerae* or *Klebsiella pneumoniae*, may have been

51 through a similar stage before accumulating diversity and that this diversity acted to generate barriers

52 to recombination, either directly or via selective pressure to reduce recombination rates between

53 genetically divergent lineages.

54

55 Specifically, we use pairwise genetic distances between strains calculated from core genome

56 sequences to estimate the recombination scaled effective population size parameter $N_e r$, where N_e is

57 the effective population size and r is the recombination rate per site per generation, an approach

58 briefly introduced by Cui et al [8]. Here, we test the method using simulated genomic datasets and

59 find that it provides accurate estimates in constant size populations for values of the scaled

60 recombination rate R (see below) of 5 or more, and responds to changes in population size or structure

61 far more quickly than estimates of N_e based on nucleotide diversity. We apply the method to real

62 genomic datasets from 21 species and find that some common species, such as *Escherichia coli*, have

63 strikingly low estimates of $N_e r$, implying that recombination is inefficient in distributing diversity

64 across the species, while for others the fit of model to data is poor, highlighting deviations from the

65 assumption of a freely recombining population. Only datasets from *V. parahaemolyticus* and

66 *Helicobacter pylori* give $N_e r$ estimates greater than 100. The former has had a recent increase in

67 population size, whereas the latter experienced repeated bottlenecks associated with geographical

68 spread of its human host, implying that neither population is close to demographic equilibrium.

69

70 **Inference approach**

71 Population genetics is a century-old discipline that provides a powerful set of theoretical and

72 statistical inference tools with which to interpret patterns of genetic variation between closely related

73 organisms. Central to the theory is the concept of a population, which in outbreeding eukaryotes is a

74 set of organisms that share a common gene pool. The simplest models assume panmixia which means

75 random mating of all individuals within a single closed population [9]. In most animals and plants

76 mating is structured by geography, however even low levels of migration between locations, of the

77 order of one individual per generation, can prevent differences from accumulating between local

78 populations, making random mating a reasonable first approximation for many outbreeding species.

79 Under neutral theory [10], the expected level of genetic diversity π depends on the per generation

80 mutation rate μ and the effective population size N_e, which is the number of individuals contributing

81 to the gene pool in each generation [11].

82

83 Outbreeding eukaryotes receive genetic material from the gene pool when they are born and

84 contribute to it when they reproduce. Individuals are more similar to immediate relatives than they are

85 to other members of the population, but the proportion of the genome shared by descent from

86 particular ancestors decays rapidly with each successive generation, declining from 1/2 for full

87 siblings to 1/8 for first cousins and 1/32 for second cousins. In small random samples from

88 populations with more than a few hundred individuals, shared recent ancestry is rare and can be

89 neglected for many types of analysis.

90

91 Bacteria reproduce by binary fission and only receive material from the gene pool or contribute to it

92 via homologous or non-homologous recombination [12]. Many cell divisions can take place between

93 consecutive recombination events, and typically recombination only affects a small fragment of the

94 genome. These properties mean that the concept of a gene pool or a population is less straightforward

95 to define than for outbreeding organisms. It is still possible to estimate the effective population size

96 N_e from the average nucleotide diversity π, based on the standard assumptions of population genetic

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97   theory, but the assumptions are both less reasonable and harder to test for bacteria than for

98   outbreeding eukaryotes, in which population boundaries can be delineated empirically using well

99   established methods [13]. For example, nucleotide diversity in a species like *E. coli* can be estimated

100   for clonal lineages, phylogroups, species or for the *Escherichia* genus as a whole. These choices lead

101   to very different values for π and hence for N_e and it is not obvious a priori which is most meaningful.

102   All methods lead to estimates of effective population size that are many orders of magnitude smaller

103   than the census number of bacteria [14].

104

105   Here, we take a different approach which is to estimate a scaled version of the effective population

106   size, $N_e r$, where r is the per generation rate at which a given site recombines. Note in particular that

107   this parameter r is the product of the per initiation site recombination rate ρ and mean tract length δ

108   used in many bacterial recombination models [15, 16]. High values of $N_e r$ indicate that the population

109   structure is similar to that of outbreeding eukaryotes. Informally, in eukaryotic population each

110   individual is the product of a separate meiosis and therefore genetically distinct. N_e is the number of

111   genetically distinct individuals that contribute in each generation, which is typically in the thousands

112   or millions. $N_e r$ is designed to measure an analogous quantity in bacteria, namely the number of

113   genetically distinct organisms that contribute to the future bacterial gene pool. To this end, time is

114   rescaled; N_e measures the rate of genetic drift per bacterial generation, while $N_e r$ measures the

115   genetic drift in proportion to the time it takes for strains to become distinct from their ancestors by

116   importing DNA by homologous recombination.

117

118   The recombination rate of *V. parahaemolyticus* is $r = 1.7 \times 10^{-4}$ per site per year [17]. After T years of

119   evolution, the expected proportion of recombined genome is e^{-rT}, so that it takes around 4,000 years

120   on average for half of the genome to recombine. Half of this time is approximately equivalent to a

121   eukaryotic generation in the sense that two strains that shared a common ancestor 2,000 years ago will

122   be about as related as siblings. This represents a very different time scale from that assumed based on

123   bacterial generations. For example, *V. parahaemolyticus* is capable of replicating in less than ten

124   minutes in appropriate conditions [18].

125

126   We estimate $N_e r$ using the pairwise genetic distances between strains, based on a number of

127   simplifying assumptions which are likely to hold true in freely recombining bacterial populations but

128   may break down in species where recombination rates are low or genetic exchange is structured by

129   geography or lineage. Specifically, the calculations assume that recombination introduces many more

130   substitutions than mutation, happens at the same rate throughout the genome and that each

131   recombination event introduces unrelated DNA from the population into the imported stretch. If

132   unrelated strains differ on average at $d_{\text{unrelated}}$ nucleotides throughout the alignment, then the

133   expected number of SNPs distinguishing strains with a common ancestor at time T in the past is

134    $d = d_{\text{unrelated}} \left(1 - e^{-2rT}\right).$

135

136   To use these times to estimate the effective population size, we assume that the genealogy of clonal

137   relationships is generated by a coalescent model with a constant population size N_e [19, 20]. This

138   model generates expectations for the times in the past at which common ancestors of strains in a

139   sample existed. Specifically, for a sample of n strains, there are $n - 1$ coalescent nodes. The age of

140   the most recent node corresponds to the common ancestor of the two most closely related strains in

141   the sample, while the $(n - 1)^{\text{th}}$ node corresponds to the common ancestor of all the strains in the

142   sample.

143

144   Coalescent theory implies that the expected time in the past at which the mth most ancient coalescent

145   event occurs is $T_m = 2N_e \left(\frac{1}{m} - \frac{1}{n}\right)$. These times can be converted into expected genetic distances d_m

146   using the formula in the previous paragraph. We use the UPGMA algorithm to obtain $n - 1$

147   coalescent distances from the $n(n - 1)/2$ pairwise genetic distances between strains and find the

148   values of $N_e r$ and $\hat{n}$ (effective sample size, see below) that gives the best fit between observed and

149   expected distances for the $n - 1$ coalescent nodes.

150

151   Note that for bacteria with high recombination rate, the genome is likely to have been scrambled up

152   sufficiently that strains will have inherited little or no DNA by direct descent from the common

153   ancestor of the entire sample. This means that the genetic distances expected for the oldest coalescent

154   events plateau at $d_{\text{unrelated}}$. In graphical representations, it is convenient to show the coalescent events

155   in chronological order with the oldest first, to aid comparisons between datasets with different sample

156   sizes.

157

158   The model assumes that the strains are randomly sampled from a homogeneous population at a single

159   time point, but pathogenic clones, epidemic outbreaks or strains from specific locations are often

160   overrepresented in real data, leading to oversampling of very closely related isolates, relative to their

161   frequency in the global population [7, 21]. Therefore, in addition to $N_e r$ we estimate a second

162   parameter $\hat{n}$ called the effective sample size, which is an estimate of the number of strains remaining

163   when over-sampled clonally related strains are removed. For simulated data where sampling is

164   random, $\hat{n}$ is correctly estimated to be very close to number of strains in the sample, so this additional

165   parameter makes little difference to the inference. For real data, estimating this additional parameter

166   often improves the qualitative model fit considerably.

167

168   **Results**

169   ***$N_e r$ can be estimated accurately for simulated data***

170   Fig. 1 illustrates the effect of varying the recombination rate in genomes simulated using FastSimBac

171   [22]. The simulations include 200 genomes of length 2 Mb under a coalescent model with constant

172   effective population size N_e. We fix the recombination tract length $\delta = 1,000$ and vary the rate of

173   initiation of recombination events ρ per $2N_e$ generations (0.001 to 0.1), so that the scaled

174   recombination rate $R = \frac{\delta \rho}{2N_e}$ varies by two orders of magnitude, from 0.5 to 50. We also fix the rate of

175   initiation of recombination event ρ (0.01) and vary the recombination tract length δ (100 to 10,000).

176   Both methods give similar results, for low scaled recombination rates, the phylogenetic tree is highly

177 structured but becomes progressively bushier as R increases, and gives the impression of being a

178 nearly perfect star for $R = 50$. For high R, the estimated $N_e r$ is close to R and the observed genetic

179 distances are well-fit by the model. For lower R, the fit is less good and the estimate of R provided by

180 $N_e r$ is also less accurate, although it remains of the right order. For these parameter values, the size

181 and shape of the phylogenetic tree are highly variable between runs, and the poorer fit reflects this

182 stochasticity as well as greater inaccuracy in the approximations made in converting between genetic

183 distances and coalescent times.

184

185 Further simulations of more complex scenarios (Supplementary Fig. 1) show that $N_e r$ estimates

186 reflect the current demography of the population more closely than estimates based on π, which is

187 more influenced by past demography and migration to and from different demes. Supplementary Fig.

188 1a shows the effect of a population expansion on estimates of $N_e r$. At time t, a single population with

189 scaled recombination rate $R = 5$ splits into two. The blue population maintains the ancestral

190 population size while the red population becomes 10 times larger. At time $t + 0.05$ the red population

191 is only marginally more diverse than the blue one but its estimated value of $N_e r$ is 6 fold higher. The

192 fit of observed and expected genetic distances is poor for the red population, reflecting the inaccuracy

193 of the modelling assumption that there is a single unchanging population size. At time $t + 0.2$ the

194 estimate of $N_e r$ is close to its true post-split value for both populations and the model fit is

195 substantially improved. Merging data from the two populations gives intermediate estimates of $N_e r$.

196

197 Supplementary Fig. 1b shows the effect of a population size reduction. A single population with $R =$

198 50 splits into two, with the blue population remaining unchanged while the red population undergoes

199 a 10 fold reduction in size. As in Supplementary Fig. 1a, the model fit for the red population is poor

200 immediately after the split but quickly improves, with the estimate of $N_e r$ approaching the correct

201 value of 5, while the nucleotide diversity of the population reduces much more slowly. Supplementary

202 Fig. 1c shows the effect of symmetric migration between two populations with a 10 fold difference in

203 effective population size. Migration has a large effect on nucleotide diversity, especially for the

204   smaller population, but has little effect on estimates of $N_e r$. Overall, these results show that our

205   inference approach provides accurate estimates of the recombination-scaled effective population size

206   for simulated data and that deviations between observed and expected genetic distances can be

207   informative about deviations from model assumptions.

208

209   **Application to *V. parahaemolyticus* genomes**

210   We first applied the method to the 1,103 *V. parahaemolyticus* genomes described in Yang et al [17].

211   For this dataset, we obtained estimates of $N_e r = 484$ and $\hat{n} = 471$ (Fig. 2a). $\hat{n}$ is less than half of the

212   sample size principally because a large fraction of the isolates in the sample belong to pandemic

213   clonal lineages responsible for large numbers of human infections. Both lineages have most recent

214   common ancestors within the last few decades and are likely to represent a very small fraction of the

215   global population of *V. parahaemolyticus*. The genetic distances fit the model well except that the 33

216   oldest coalescent events, on the left hand side of the plot are larger than $d_{\text{unrelated}}$. The discrepancy is

217   largely due to population structure within the species, since the fit is better, although still not perfect

218   when analysis is restricted to the 944 isolates from the VppAsia population (Fig. 2a).

219

220   As described by Yang et al., the sample of *V. parahaemolyticus* is subdivided into 4 modestly

221   differentiated populations, likely due to historical barriers to migration between oceans [17]. For the

222   VppAsia population $N_e r$ was estimated to be 453 which is similar to that for the dataset as a whole,

223   while other populations have substantially smaller values. These differences are not simply due to a

224   larger sample size since estimates based on subsets of the VppAsia data are consistently greater than

225   200 (Fig. 2b). Sampling strategy does make some difference, since estimates are lower for a dataset

226   consisting only of clinical strains than of shellfish, fish or all non-clinical isolates (Fig. 2c). This

227   difference presumably reflects variation in disease causing potential, which results in samples of

228   clinical isolates not representing the full diversity of clonal lineages within the species.

229

230   **Application to multiple bacterial species**

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231   We also applied the method to a survey of other bacteria for which large numbers of genomes are

232   publically available (Supplementary Fig. 2). *V. cholerae* has an estimate of $N_e r$ of 29, while *Vibrio*

233   *vulnificus* has a value of 43. Although the sample sizes available are relatively limited, in contrast to

234   the Asian population of *V. parahaemolyticus*, the genetic distances do not show a clear plateau

235   corresponding to a single value for $d_{\text{unrelated}}$ and while there are a continuous range of coalescent

236   distances, the overall fit between model and data is poor. Thus, although the other *Vibrio* species in

237   our dataset recombine frequently they are far from being panmictic.

238

239   *H. pylori* has the largest estimated value of $N_e r$ of all datasets we analysed. *H. pylori* is characterized

240   by extremely high rates of recombination during mixed infection of the human stomach, with 10% or

241   more of the genome recombined during a single infection [23] and linkage disequilibrium decreases

242   much more rapidly as a function of genetic distance than for all other species, including *V.*

243   *parahaemolyticus* (Fig. 3a). Although the tree is approximately star-like, the fit of the model is not

244   perfect, with no clear plateau for a single value of $d_{\text{unrelated}}$, due to the complex geographical

245   population structure of the species [24].

246

247   Amongst the other species, $N_e r$ varies from 1 for *Chlamydia trachomatis* to 88 for *Salmonella*

248   *enterica*. The overall fit of the model varies considerably between species (Supplementary Fig. 2) and

249   is typically worst for the oldest coalescent events, as examined in more detail above for *V.*

250   *parahaemolyticus*. Our estimated $N_e r$ values are correlated with the linkage disequilibrium statistic r^2

251   measured between distant makers at pairwise distance of 3 kb (Fig. 3b). However, $N_e r$ shows no

252   correlation with nucleotide diversity of synonymous sites π_{syn} (Fig. 3c) and r^2 at pairwise distance of

253   3 kb also shows no correlation with π_{syn} (Fig. 3d).

254

255   **Discussion**

256   **Structure of genetic diversity in 21 bacterial species**

257   In bacteria, adaptation to diverse environmental challenges should be most effective in species where

258   realized recombination rates are high enough to thoroughly mix up genetic variation (panmixia), since

259   this creates the largest possible pool of genotypes on which natural selection can act. High

260   recombination can also make it easier for researchers to detect the imprint of natural selection, a

261   feature exploited by Cui et al. 2015, 2018 [8, 25] in investigating coadaptation in *Vibrio*

262   *parahaemolyticus*. However, despite the utility of well-mixed gene pools, both to the species that have

263   it and to the researchers studying it, it appears to be rare in bacteria for which large numbers of

264   genomes are currently available.

265

266   The genetic structure of bacteria species depends on the interplay of many different processes,

267   including changes in population size over time, geographical and ecological subdivision and the

268   complex biology of genetic exchange which takes place via conjugation, transformation and

269   transduction. As a result no single parameter summarizes the effect of recombination in breaking

270   down linkage disequilibrium. Furthermore, available genomes rarely come close to being a random

271   sample of the bacterial population from which they are taken.

272

273   Here we have used two summary statistics of the effectiveness of recombination; a non-parametric

274   measure of long-range linkage disequilibrium, r^2 between markers 3 kb apart on the genome, and a

275   parametric approach estimating the composite population genetic parameter $N_e r$. Informally, high

276   values of $N_e r$ implies that recombination has generated new clonal complexes quickly compared to

277   the rate at which genetic drift (proportional to $1/N_e$) removes them, with the result there are many

278   distinct clonal-complexes segregating in the population. In data simulated according to a coalescent

279   model with recombination of short tracts, estimated values of $N_e r$ are close to the true values for

280    $N_e r > 2$.

281

282   In our survey of 21 species from which large numbers of genomes are available, our two summary

283   statistics are strongly but incompletely correlated ($R^2 = 0.69$, $P < 0.01$, Fig. 3b). According to both

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284   statistics, two species are clear outliers, with estimates of $N_e r = 453$ for the Asian population of *V.*

285   *parahaemolyticus* and 1,976 for the hpEurope population of *H. pylori,* and the estimated value of $N_e r$

286   was lower than 100 in the other 19 species, which are therefore far from being panmictic.

287

288   Estimated $N_e r$ or r^2 are uncorrelated with the nucleotide diversity of synonymous sites π_{syn} (Fig. 3c,

289   Fig. 3d). For example, *V. parahaemolyticus* has similar diversity to *V. cholerae* and much lower

290   diversity than *V. vulnificus*, both of which have estimated $N_e r$ lower than 50. Furthermore, estimates

291   of $N_e r$ vary over a factor of 1,000, while π varies only by a factor of 10.

292

293   **Mechanisms by which high diversity can create barriers to recombination**

294   Since panmixia is possible within bacterial populations and should facilitate genetic adaptation to the

295   widest possible range of niches available to the species, it raises the question why it is not widespread.

296   There are several bacterial species in our sample which survive well in the environment, have

297   effective global dispersal, enormous census population sizes and high recombination rates, such as *E.*

298   *coli, Campylobacter jejuni* and *K. pneumoniae*. Species with these characteristics are the most

299   obvious candidates to be panmictic, whereas *V. parahaemolyticus* thrives only in warm brackish

300   waters and has oceanic gene pools, implying historical limits on its dispersal [17], and *H. pylori* only

301   survives in human stomachs and shows geographic differentiation associated with historical

302   migrations of its host [26].

303

304   Neutral population genetic models imply that, all else being equal, bacteria with higher census

305   population sizes should have higher $N_e r$ as well as higher nucleotide diversity, which is proportional

306   to $2N_e \mu$ at equilibrium. However, across our 21 species, there is no correlation between the two

307   statistics. We propose that the absence of a correlation occurs because high diversity tends to

308   supresses recombination. There are a number of mechanisms by which this suppression can occur that

309   have been described in the literature and we do not attempt to reach a conclusion about which are in

310   fact most important in nature.

12

311

312   For example, in Cui et al [25], we observe nascent boundaries to genetic exchange between lineages

313   of *V. parahaemolyticus* associated with differentiation into ecological types. This kind of genetic

314   differentiation is more likely to be common, and more likely to lead to barriers to exchange in more

315   diverse bacterial populations, which tend to have more diverse accessory genomes [27], and might

316   also have a larger number of distinct ecological niches. Such barriers can result in speciation [21, 28]

317   but can also lead to genetic structuring within a single species, such as host-specific gene pools found

318   in *C. jejuni* [29].

319

320   Another mechanism is changes in the pattern or rate of recombination. Recombination requires

321   homology between donor and recipient DNA to be recognized by the cellular machinery and, in

322   addition, most species have a mismatch repair system, which aborts the process if there are too many

323   differences. Therefore, as homology decreases, so will recombination [23, 28, 30, 31]. Mismatch

324   dependent recombination is likely to be the main reason why *E. coli* has an estimated value of $N_e r$ of

325   only 12, implying that recombination is ineffective in reassorting variation across the whole species

326   [32].

327

328   Finally, a genetic dependence of recombination rates on diversity might arise from effects of epistasis

329   in constraining realized recombination. Simulations of facultatively sexual organisms have shown

330   that selection on multiple interacting loci can lead to populations being dominated by small numbers

331   of clones, even in the presence of frequent recombination [33]. These simulations also show that a

332   phase transition from panmixia can take place due to a decrease in recombination rate or even an

333   increase in population size.

334

335   ***Vibrio parahaemolyticus* and *Helicobacter pylori* are far from demographic equilibrium**

336   If, as we propose, suppression of recombination due to high diversity is a general phenomenon in

337   bacteria, then the existence of populations with high $N_e r$ might seem paradoxical, because high N_e

338   leads to high diversity which should suppress r. However this argument only holds in populations

339   where N_e has been high for long enough for diversity to accumulate and do not apply if high N_e is a

340   recent phenomenon on an evolutionary timescale.

341

342   In fact, neither the Asian population of *V. parahaemolyticus* nor *H. pylori* are close to demographic

343   equilibrium. As proposed in Yang et al [17], the Asian population of *V. parahaemolyticus*, VppAsia,

344   has spread within the last few decades due to human activity but has an ancestral range restricted to

345   coastal waters from India to Japan. The other *V. parahaemolyticus* populations that we have sampled,

346   with different ancestral ranges, have smaller estimated $N_e r$, perhaps because they have smaller or less

347   fecund ranges or experience greater competition or more frequent demographic bottlenecks.

348

349   Crucially, it seems that the ancestral population size of *V. parahaemolyticus* as a whole was smaller

350   than currently found in VppAsia and more similar to that in the other populations. First, the site

351   frequency spectrum of VppAsia, but not the other populations, is out of equilibrium and is

352   approximately consistent with a demographic scenario in which the effective population size

353   increased by a factor of ten 15,000 years ago (Supplementary Fig. 3). One possible scenario is that the

354   end of the last ice age around 11,000 years ago created a habitat that existed till the present and that

355   the population expansion implied by the site frequency spectrum is due to a demographic expansion at

356   the end of the ice age.

357

358   Secondly the level of synonymous nucleotide diversity in the population is 30-fold lower than

359   expected at demographic equilibrium. r/μ, the number of recombinant sites for each mutant site, has

360   been estimated for the species to be around 313 [17]. Note that this parameter r/μ is not the same as

361   the parameter r/m often used in the literature [6] because the former considers all recombined sites

362   whereas the latter considers only recombinant sites that are substituted. If nucleotide diversity reached

363   an equilibrium, consistent with the current value of $N_e r$ of VppAsia, this would predict a value of

364     $2N_e\mu = 2N_e r \left(\frac{\mu}{r}\right) = 2 \times 453 \times \left(\frac{1}{313}\right) = 2.9.$ At equilibrium in a neutrally evolving population,

365   with sites evolving according to the Jukes-Cantor model, π is predicted to be equal to $\frac{3}{4} \times$

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366    $\left(1 - e^{-\frac{8N_e\mu}{3}}\right) = 0.73$, and therefore most synonymous sites should differ between individuals. This is

367   far from the current value of 0.024 or indeed diversity levels in any well-characterised bacterial

368   species. Our simulation results show that estimates of $N_e r$ respond much more quickly to changes in

369   demography than π, implying that diversity levels would be likely to continue a slow but steady rise if

370   the effective population size remains constant (ignoring the changes in population structure caused by

371   human activity in recent decades).

372

373   The equivalent argument is more complex in *H. pylori* due to its geographic population structure but

374   levels of variation are clearly far from demographic equilibrium, as illustrated by the substantial

375   variation between populations that reflects bottlenecks associated with historical human migration

376   rather than current population sizes [26]. These results therefore suggest that if effective population

377   sizes remain continuously high in either species, then diversity would increase. We propose that in

378   this case, barriers to recombination would evolve, perhaps by one of the mechanisms described above.

379

380   Despite decades of study by population geneticists, the factors determining the amount of diversity

381   within species are still poorly understood, for example with much lower variation in nucleotide

382   variation π between species than is expected based on variation in census population size in many

383   domains of life [34, 35]. Our results suggest that patterns of genome variation in bacteria are likely to

384   be dynamic, with ecological and genomic differentiation, barriers to gene flow and demographic

385   factors interacting with each other in complex ways to alter both the level of genetic diversity within

386   the species and the way it is partitioned, to produce the wide variety of bacterial population structures

387   that are observed.

388

389   **Material and methods**

390   **Genomic datasets used in analysis**

391   Because our inference approach of $N_e r$ hypothesizes that recombination drives the genetic variation

392   other than mutation, we firstly selected 27 bacterial species, in which r/m values (ratio of nucleotide

15

393   changes resulted from recombination relative to point mutation) of them were greater than one based

394   on previous multi-locus sequence typing data estimation [6]. We then counted the number of

395   assembled genomes of them in NCBI database, and found that the genome numbers of 21 bacterial

396   species were greater than 100, which were used in further analysis, including *Bacillus thuringiensis,*

397   *Burkholderia pseudomallei, Campylobacter jejuni, Chlamydia trachomatis, Enterococcus faecalis,*

398   *Escherichia coli, Flavobacterium psychrophilum, Haemophilus influenzae, Helicobacter pylori,*

399   *Klebsiella pneumoniae, Legionella pneumophila, Leptospira interrogans, Neisseria meningitidis,*

400   *Porphyromonas gingivalis, Pseudomonas syringae, Salmonella enterica, Streptococcus pneumoniae,*

401   *Streptococcus pyogenes, Vibrio cholerae, Vibrio parahaemolyticus* and *Vibrio vulnificus.*

402

403   For species with more than 500 genomes in NCBI database, such as *E. coli* (>14,000 assembled

404   genomes), 500 genomes were randomly selected to reduce the amount of calculation. Each genome

405   was aligned against the reference genome of the corresponding species (Supplementary Table 1) using

406   MUMmer [36] to generate the whole genome alignments and identify SNPs in core genomes (regions

407   presented in all isolates) as previously described [8, 17]. Only genomes with genome-wide coverage >

408   70% (compared to reference genome) were used in further analysis. SNPs located in repetitive regions

409   were removed, and the filtered bi-allelic SNPs sets were used to construct the Neighbour-joining (NJ)

410   tree of each species. Strains located on the extremely long branches of the NJ tree and strains

411   belonged to clonal groups were manually removed, finally resulting in a dataset of 6,355 genomes

412   (56-1,103 genomes for each species). The accession numbers of genomes used (excluding *H. pylori*

413   and *V. parahaemolyticus*) were listed in Supplementary Table 1, and the whole-genome alignments

414   were available in the figshare data repository (https://figshare.com/s/3f9d04a8229f30dd785b).

415

416   **SNP calling, phylogeny reconstruction and LD decay calculation**

417   The SNP dataset of 278 *H. pylori* (hpEurope population) and 1,103 *V. parahaemolyticus* genomes

418   were reused from previous studies [17, 37]. The SNPs of the left 4,974 genomes were recalled using

419   same pipelines as described above. Totally 17,875-462,214 bi-allelic SNPs were identified separately

420   for 21 bacterial species, which were used in further analysis, including Neighbour-joining tree

421   construction, pairwise SNP distance calculation, LD r^2 value calculation, and $N_e r$ estimation as

422   previously described [8]. The Neighbour-joining trees were constructed using the software Treebest

423   (http://treesoft.sourceforge.net/treebest.shtml) based on concatenated SNPs. Haploview [38] was used

424   to calculate the LD r^2, and the maximum comparison distance was set to 30 kb.

425

426   **Simulation**

427   The software FastSimBac [22] was used to generate the simulated bacterial populations under

428   different hypothetical evolution scenarios, including a constant population with different scaled

429   recombination rate R (Fig. 1), and the changing populations with population expansion

430   (Supplementary Fig. 1a), reduction (Supplementary Fig. 1b) and migration (Supplementary Fig. 1c).

431   All the simulated genome length was set to 2 Mb, mutation rate was fixed at 0.01 (per site per $2N_e$

432   generations). The detailed parameters used in simulation were listed in Supplementary Table 2.

433

434   **Recombination scaled effective population size estimation**

435   For a freely mixing population in which recombination drives diversification, neutral theory predicts

436   that $N_e r$ is in proportion to genealogical coalescent rate, and the expected coalescent curves can be

437   estimated based on the formula $d = d_{\text{unrelated}}(1 - \exp(-4N_e r \left(\frac{1}{m} - \frac{1}{n}\right)))$ as mentioned in previous

438   work [8]. In the formula, d is the expected pairwise genetic divergence, $d_{\text{unrelated}}$ is the median

439   pairwise SNP distance of a population, n is the number of individual strains and m is the index of the

440   ancestral node along the coalescent tree of n strains. Coalescent curves can be estimated using the

441   UPGMA algorithm based on the pairwise SNP distance. By fitting the expected and observed

442   coalescent curves with least square method to search for the optimal parameters, we found the optimal

443   values of $\hat{n}$ (effective sample size) and $N_e r$ that were used in further analysis. $\hat{n}$ was an estimate of

444   the number of strains remaining when over-sampled clonally related strains are removed.

445

446   **Site frequency spectrum estimation**

447 The minor allele frequency (MAF) of each SNP locus in a SNP matrix was calculated and then the

448 frequency of SNP positions at each MAF level were counted to generate the site frequency spectrum

449 (SFS) for each dataset. To get a comparable result, the SFS showed in each Figure or panel was

450 calculated based on same sample size. In Supplementary Fig. 3a, the merged population was

451 generated by randomly selecting 100 genomes from each of Pop1 and Pop2. In Supplementary Fig.

452 3b, the same number of genomes as in the real genome dataset were simulated to show populations

453 with different evolution scenarios.

454

462

Author Contributions

464 D. F., Y. C., and R. Y. designed the study and coordinated the project; C. Y., Y. C., X. D., and D. F.

465 analyzed the data; D. F. wrote the manuscript. All authors approved the final version of the

466 manuscript.

467

Competing Financial Interests statement

469 None

470

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560

561   **Figure legends**

562   **Figure 1. Recombination scaled effective population size ($N_e r$) estimation of simulated constant**

563   **populations under different scaled recombination rate.** The panels indicated populations with

564   scaled recombination rate *R* of 0.5 (a), 1 (b), 5 (c), 25 (d), 50 (e). From top to bottom, indicating the

565   NJ trees, distribution of pairwise SNP distance between individuals and observed and expected

566   coalescence curves. The dashed line of middle and bottom panels indicated the median SNP distance

567   between individuals. The red points in the bottom panel indicated the expected distances between $n -$

568   1 coalescent nodes, the blue triangles indicated the observed distances estimated from pairwise SNP

569   distances using the UPGMA algorithm.

570

571   **Figure 2. Recombination scaled effective population size ($N_e r$) estimation of *V.***

572   ***parahaemolyticus*.** (a) $N_e r$ estimation of all the samples and four populations (VppAsia, VppX,

573   VppUS1 and VppUS2) of *V. parahaemolyticus*. Layout and colors are the same as in Figure 1. (b)

574   $N_e r$ estimation of VppAsia population based on different sample sizes. 100-500 genomes were

575   randomly selected from total 944 VppAsia genomes, 10 repeats were performed for each sample size

22

576   to create the boxplot. (c) $N_e r$ estimation of VppAsia population based on different types of samples.

577   Points and lines show observed and expected coalescence curves, respectively.

578

579   **Figure 3. Correlation between $N_e r$, linkage disequilibrium (LD) statistic r^2 and nucleotide**

580   **diversity of synonymous sites.** (a) LD decay of 21 bacterial species. The maximum comparison

581   distance was set to 30 kb. The vertical dashed line indicated the LD r^2 values at pairwise distance of 3

582   kb, which were used in panel b and d. Line colours indicated the estimated $N_e r$ values. (b).

583   Correlation between $N_e r$ and LD r^2 values. (c) Correlation between $N_e r$ and nucleotide diversity of

584   synonymous sites π_{syn}. (d) Correlation between LD r^2 values and nucleotide diversity of synonymous

585   sites π_{syn}. Point colours in panel b-d indicated the estimated $N_e r$ values.

23