A non-adaptive demographic mechanism for genome expansion in *Streptomyces*

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Abstract

The evolution of microbial genome size is driven by gene acquisition and loss events that occur at scales from individual genomes to entire pangenomes. The equilibrium between gene gain and loss is shaped by evolutionary forces, including selection and drift, which are in turn influenced by population demographics. There is a well-known bias towards deletion in microbial genomes, which promotes genome streamlining. Less well described are mechanisms that promote genome expansion, giving rise to the many microbes, such as *Streptomyces*, that have unusually large genomes. We find evidence of genome expansion in *Streptomyces* sister-taxa, and we hypothesize that a recent demographic range expansion drove increases in genome size through a non-adaptive mechanism. These *Streptomyces* sister-taxa, NDR (northern-derived) and SDR (southern-derived), represent recently diverged lineages that occupy distinct geographic ranges. Relative to SDR genomes, NDR genomes are larger, have more genes, and their genomes are enriched in intermediate frequency genes. We also find evidence of relaxed selection in NDR genomes relative to SDR genomes. We hypothesize that geographic range expansion, coupled with relaxed selection, facilitated the introgression of non-adaptive horizontally acquired genes, which accumulated at intermediate frequencies through a mechanism known as genome surfing. We show that similar patterns of pangenome structure and genome expansion occur in a simulation that models the effects of population expansion on genome dynamics. We show that non-adaptive evolutionary phenomena can explain expansion of microbial genome size, and suggest that this mechanism might explain why so many bacteria with large genomes can be found in soil.

Importance

Most bacterial genomes are small, but some are quite large, and differences in genome size are ultimately driven by the interplay of gene gain and loss dynamics operating at the population level. The evolutionary forces that favor genome size reduction are well known, but less understood are the forces that drive genome expansion. It is generally assumed that large genomes are adaptive because they favor metabolic versatility. However, we find evidence in *Streptomyces* for a non-adaptive mechanism of genome expansion driven by horizontal gene transfer. We hypothesize that historical range expansion decreased the strength of selection acting these genomes. Relaxed selection allowed many newly acquired genes (which would normally be lost to deletion) to accumulate, leading to increased genome size. *Streptomyces* have large genomes that contain a remarkable diversity of antibiotic producing gene clusters, and genome expansion has likely contributed to the evolution of these traits.

Introduction

Microbial genomes are extraordinarily dynamic. Genome size varies considerably, and gene content in strains of the same species can differ dramatically, giving rise to the pangenome. The pangenome concept has transformed our understanding of evolutionary processes in diverse taxa (1–4). The pangenome is the entire collection of genes in a microbial species, and is subdivided into core genes present in all strains, dispensable or accessory genes present in some strains, and strain-specific or unique genes (5, 6). Rates of gene acquisition and gene loss determine the individual genome size, and consequently, pangenome composition is shaped by evolutionary mechanisms that alter gene frequencies in microbial populations (7–9).

Genome size varies by four orders of magnitude (10^4–10^7 kb) in eukaryotic organisms and two orders of magnitude in prokaryotic organisms (from less than 150 kb in certain endosymbionts to over 10 Mb for some free-living bacteria) (10). Unlike eukaryotes, whose genomes contain large portions of non-coding DNA, prokaryotic gene content is directly related to genome size because bacterial and archaeal taxa have high coding density (11, 12). While microbial genomes are constantly in flux, deletion rates are approximately three-fold greater than rates of gene acquisition (13). Multiple factors contribute to the strong deletion bias in microbial genomes, including selection for efficiency, "use it or lose it" purging of nonessential genes, and genetic drift (14–17).

Because of this tendency towards deletion, microbial genome reduction has been examined in greater detail than genome expansion. For example, the evolutionary mechanisms driving genome reduction in obligate pathogens like *Rickettsia* and symbionts like *Buchnera* in aphids are well described (17, 18). The transition from a free-living to a host-associated lifestyle involves substantial loss of superfluous genes, and generations of vertical transmission in small asexual populations leads to gene inactivation and deletion accelerated by genetic drift (16). Alternatively, genome streamlining leads to reduction of both genome and cell size through selection for increased metabolic efficiency in free-living microbes with large populations (15). Genome streamlining is historically associated with marine oligotrophic *Pelagibacter* (14, 19) but has more recently been described for soil-dwelling *Verrucomicrobia* (20).

Large genomes are frequent among terrestrial free-living microbes, and must be the product of evolutionary forces that drive genome expansion. A common, though relatively untested, hypothesis to explain large genomes is that high environmental heterogeneity (a characteristic of terrestrial habitats) selects for metabolic versatility afforded by gene gain, and thereby drives genome expansion (21, 22). For example, massive gene acquisition and adaptation to alkaline conditions caused genome expansion in the myxobacterium *Sorangium cellulosum*, which at 15 Mb is one of the largest known bacterial genomes (23). Mechanisms of gene gain include duplication or horizontal gene transfer (HGT), and large genomes are enriched in functional genes acquired from phylogenetically distant origins (24). Much of the evolution of gene families can be attributed to HGT rather than duplication events (25, 26), and HGT is a major driver of genome expansion (27, 28). While HGT-mediated gene acquisition occurs with great frequency, microbial genomes remain relatively small, and genome size tends to be fairly conserved within a species.

Gene frequencies at the population-level are governed by selection and drift, and these evolutionary forces determine whether a newly acquired gene will be purged from the pangenome or whether it will sweep to fixation. The strength of selection and drift varies inversely, and their relative contributions are determined by a gene's selection coefficient and effective population size (N_e) (29, 30). Drift can exert large effects on populations with small N_e, but these effects decline as N_e increases and selection intensifies. Our ability to disentangle the contributions of selection and drift to pangenome dynamics are complicated by the fact that it remains difficult to estimate microbial N_e (31, 32) and to delimit microbial population and species boundaries (33–35). Another complication is that demographic models often include the simplifying expectation that N_e is invariable over time.

Rapid changes in population size are typical in the evolutionary histories of many microbial species, and fluctuations in N_e such as population bottlenecks or expansions can have profound impacts on contemporary patterns of genomic diversity. For example, the population structure for many pathogenic bacterial lineages is exemplified by episodes of rapid expansion of clonal complexes repeated across space and time (36–38). Microbial population expansions can also be linked to ecological or geographical range expansions (39–42). For instance, demographic expansion in the oral bacteria *Streptococcus mutans* coincides with the origin of human agricultural practices (41).

We find evidence for post-glacial range expansion in the genus *Streptomyces*, and these species exhibit several of the genetic characteristics described in plant and animal species whose biogeography was influenced by Pleistocene glaciation (43, 44). By examining *Streptomyces* isolated from sites across North America, we observed genetic evidence for dispersal limitation, a latitudinal gradient in taxonomic richness, and a latitudinal gradient in genetic diversity (45, 46). We also identified recently diverged sister-taxa comprising a more genetically diverse southern-derived (SDR) clade and a more homogenous northern-derived (NDR) clade, which occupied discrete geographic ranges spanning the boundary of glaciation (47). We further observed larger genomes in the northern clade compared to the southern clade.

We hypothesize that genome expansion in NDR is a consequence of demographic change driven by post-Pleistocene range expansion. Here, we evaluate the effects of historical range expansion on lineage divergence, genome size, and pangenome structure, and assess these data in the context of the genome surfing hypothesis. Genome surfing is a non-adaptive mechanism which describes the introgression of horizontally acquired genes facilitated by relaxed selection and amplified by geographic expansion (48). We hypothesize that range expansion, coupled with relaxed selection, dampened gene loss thereby facilitating an increase in non-adaptive, intermediate frequency genes in the NDR pangenome. We infer gene gain and loss dynamics by evaluating patterns of shared gene content between strains. We predict that the contribution of drift is greater in NDR compared to SDR, and determine the relative strength of selection by comparing genome-wide rates of amino acid substitution between clades. Finally, we evaluate our hypothesis by modeling population expansion under a regime of relaxed selection and ask whether these demographic conditions increase retention of horizontally acquired genes at intermediate frequencies, ultimately causing genome expansion.

Results

Streptomyces sister-taxa

We sequenced the genomes of 20 *Streptomyces* strains isolated from ecologically similar grasslands sites across the United States (Table S1, Table S2). These genomes derive from sister-taxa comprising a northern-derived (NDR) and southern-derived clade (SDR), which originate from sites spanning the historical extent of glaciation (Figure S1, see (45)). These sister-taxa represent closely related but genetically distinct microbial species. Genomes within NDR share 97.8 ± 1.3% (mean ± SD) ANI and those within SDR share 97.6 ± 0.1% (mean ± SD) ANI, while inter-clade genomic ANI is 93.0 ± 0.14% (mean ± SD). An ANI of 93–96% is typically indicative of taxonomic species boundaries (49, 50). For comparative purposes, we also sequenced the genomes of four strains that co-localized with the sister-taxa. The closest taxonomic neighbor to these 24 strains is *Streptomyces griseus* subsp. *griseus* NBRC 13350, although all strains share < 95% ANI with this type strain (Figure S1).

Genomic attributes and gene content

NDR genomes are larger (8.70 ± 0.23 Mb, mean ± SD) than SDR genomes (7.87 ± 0.19 Mb, mean ± SD), and this difference is significant (Mann Whitney U test; $P < 0.0001$) (Figure 1a). NDR genomes also have also have more orthologous protein-coding gene clusters (hereby referred to as genes) (7,775 ± 196 genes, mean ± SD) than SDR genomes (7,093 ± 205 genes, mean ± SD), and this difference is also significant (Mann Whitney U test; $P < 0.0001$) (Figure 1b). As expected, there is a strong positive correlation between genome size and gene content ($R^2 = 0.95$, $P < 0.0001$), but coding density did not differ between clades (Figure S2). SDR genomes are more genetically diverse than NDR. Nucleotide diversity (π) across conserved, single-copy core genes is greater in SDR than NDR, and this difference is significant (Mann Whitney U test; $P < 0.0001$) (see (47)). Finally, NDR genomes have slightly lower genome-wide GC content (71.50 ± 0.087%, mean ± SD) than SDR genomes (71.62 ± 0.11%, mean ± SD), and this difference is significant (Mann Whitney U test; $P = 0.017$) (Figure 1c). Shared gene content between strains correlates with genomic similarity as measured by ANI (NDR: $R^2 = 0.82$, $P < 0.0001$; SDR: $R^2 = 0.64$, $P < 0.0001$) (Figure 2). However, gene content varies more in NDR than in SDR, and there is a significant interaction between genomic similarity and clade with respect to gene content shared between strains (Table S3). This interaction comes from shared gene content between strains increasing more rapidly over recent phylogenetic timescales in NDR compared to SDR (Figure 3).

Pangenome structure and dynamics

The 24 *Streptomyces* genomes (Table S2) contain 22,055 total orthologous protein-coding gene clusters (i.e., genes), and 42% (9,285 genes) are strain-specific. All 24 genomes share 3,234 (2,778 single-copy) genes, which represent 40–48% of the total gene content per strain. While NDR has a smaller core genome than SDR (4,234 and 4,400 genes, respectively), its pangenome is larger (13,681 genes in NDR versus 12,259 genes in SDR) and contains a greater number of clade-specific genes (5,647 genes unique to NDR versus 4,308 genes unique to SDR) (Figure 3, Figure 4, Table S4).

For most microbial species, pangenome frequency distributions are U-shaped, reflecting high proportions of both strain-specific genes and core genes (51). While the pangenome structures of our *Streptomyces* sister-taxa generally conform to this shape, the NDR pangenome is enriched in intermediate frequency accessory genes relative to SDR (Figure 4). The proportion of intermediate-low frequency (i.e., present in 3–5 strains) accessory genes is higher in NDR than in SDR (19% of total genes for NDR versus 9.2% of total genes for SDR) (Table S4), and this difference is statistically significant (two proportion z-test; $P < 0.0001$). Conversely, the proportion of intermediate-high frequency (i.e., present in 6–8 strains) accessory genes is equivalent (6.9% of total genes for NDR versus 7.2% of total genes for SDR; two proportion z-test; $P = 0.26$) (Table S4).

Next, we determined if genes across different gene pools, binned according to their pangenome frequencies, differed in genetic attributes including per-gene GC content and codon usage bias. GC content differs between gene pools for both NDR and SDR pangenomes (ANOVA; $F_{3,\ 25932}$

= 267.5, P-value < 0.0001) (Figure S3). In general, GC content is greater in high frequency and core genes compared to rare and intermediate frequency genes for both sister-taxa. Codon usage bias as measured by the effective number of codons (ENC) (52) also differs between gene pools for both NDR and SDR pangenomes (ANOVA; $F_{3,\,21624}$ = 1862.7, P-value < 0.0001) (Figure S4). Rare and intermediate frequency genes exhibit less overall codon bias compared to high frequency and core genes, which tend to use codons more preferentially.

Historical population demography

Due to founder effects occurring at the edge of an expanding population, N_e is dramatically reduced during geographic range expansion (53). Consequently, relaxed selection will accompany range expansion since the contribution of selection scales directly with N_e. Based on the theory of neutral molecular evolution, which states that selection on synonymous sites is negligible (54), the ratio of non-synonymous to synonymous amino acid substitutions (K_A/K_S) reflects the relative strength of selection acting on a sequence. When assessed at the level of single-copy genes conserved between the sister taxa (2,444 genes), we observe that genome-wide K_A/K_S tends to be higher in NDR than in SDR (Figure 5), and this difference is significant (Mann-Whitney U test; P < 0.0001). This result indicates that selection is weaker and genetic drift stronger in NDR relative to SDR.

We used a population model (modified from (55)) to determine whether demographic expansion could produce increased intermediate gene frequencies and result in genome expansion. We simulated gene gain and loss events in a population undergoing exponential growth over 100 generations, and determined changes in pangenome structure and genome size. To approximate relaxed selection during the population expansion, we imposed a fitness penalty for newly acquired genes that scaled inversely with population size. At the beginning of expansion, most genes were present at high frequencies due to strong founder effects (Figure S5, top and middle). During the expansion, we observed a transient enrichment of intermediate frequency genes within the pangenome (Figure S5, top and middle). Total gene content also increased during population expansion due to relaxed selection pressure when N_e was small, which allowed for the persistence of newly HGT-acquired genes. Genome size stabilized when N_e reached maximum size, and selection pressure balanced HGT-mediated gene gain with simultaneous gene loss (Figure S5, bottom).

Discussion

We have hypothesized that the biogeography of our *Streptomyces* sister-taxa is explained by historical demographic change driven by geologic and climatic events that occurred in the late Pleistocene (46, 47). Following the last glacial maxima, North American plant and animal species rapidly colonized glacial retreat zones, and the genetic consequences of post-glacial expansion are well documented and include northern-ranged populations with low diversity that established vast geographic extent (43, 44). We hypothesize that the recent common ancestor of NDR and SDR inhabited southern glacial refugia prior to the last glacial maxima (LGM). Post glaciation, NDR dispersed northward and colonized the latitudinal range it occupies today (see (46)). We previously described patterns of gene flow, genomic diversity, and ecological adaptation in these sister-taxa, with both adaptive and non-adaptive processes likely reinforcing lineage divergence (47). Here, we evaluate the outcomes of historical range expansion on sister-taxa pangenome structure and genome size.

Expanding populations experience repeated founder effects as individuals along the leading edge disperse and colonize new landscapes, creating spatial patterns of genetic diversity akin to genetic drift (53). Allele surfing, or gene surfing, is a non-adaptive mechanism that propagates rare alleles along an expanding edge such that neutral, or even deleterious, variants 'surf' to higher frequencies than would be expected under population equilibrium (56–58). When applied to expanding microbial populations, gene surfing can facilitate genome surfing, a neutral mechanism acting at the pangenome level that causes rare genes to surf to higher frequencies independent of natural selection (48). Below, we outline how historical range expansion and genome surfing could give rise to genome expansion in *Streptomyces*.

Genome surfing is most likely to occur in microbial populations with intermediate levels of dispersal and in taxa capable of HGT. Bacteria in the genus *Streptomyces* are ubiquitous in soil and produce desiccation and starvation resistant spores which are easily disseminated (59), making them ideal for studying patterns of biogeography dependent on dispersal limitation. Rates of HGT in *Streptomyces* are among the highest estimated across a range of bacterial species (60–62). In many instances, HGT events occurred in ancestral lineages creating patterns of shared genetic ancestry and reticulate evolution in many extant *Streptomyces* species (63). We previously observed a distance decay relationship between sites up to 6,000 km apart, indicative of dispersal limitation at intermediate spatial scales that allows detection of geographic patterns of diversity across the sampled range (45, 46). We also found evidence of restricted gene flow between the core genomes of NDR and SDR (47).

Since NDR and SDR sister-taxa share a recent common ancestor (Figure S1), they must also share a common ancestral genome size. Hence, differences in genome size accompanying lineage divergence resulted from either genome expansion in NDR or genome reduction in SDR. Given that changes in genome size are ultimately the result of gene gain and loss, we first evaluated differences in shared gene content between NDR and SDR strains. We find greater variability in shared gene content in NDR compared to SDR (Figure 2, Figure 3). This result suggests relative gene content stability for SDR and gene content instability for NDR, most notably in recent phylogenetic history (Figure 3). Likewise, the pangenome of NDR exceeds that of SDR by over 1,000 genes. Evidence suggests that during range expansion, founders at the expansion edge disperse into new habitats and acquire genes from local gene pools asymmetrically at unequal rates, and gene flow is almost exclusively from local to invading genomes (64). These data are consistent with the observation that that NDR has a larger, more diverse, and more dynamic pangenome than SDR due to introgression from local gene pools. Regardless of their origin, most novel horizontally-acquired genes are neutral or nearly neutral (65). In most situations, selection will balance gene gain with gene deletion, and genome size will remain relatively constant.

Genetic diversity in individuals at the leading edge of an expanding population is dramatically reduced, and their genomes experience relaxed selection pressure due to consecutive population bottlenecks and low N_e (66). We find that NDR has lower genetic diversity (47) and higher rates of K_A/K_S across its core genome relative to SDR (Figure 5), which is consistent with the prediction that NDR has experienced a period of relaxed selection relative to SDR. A positive correlation is observed between GC content and selection pressure on microbial genomes (67, 68), and genome expansion in *Chlamydia* has been linked to relaxed selection resulting in a decrease in genome-wide GC content (69). We likewise observe a decrease in genome-wide GC content in NDR relative to SDR (Figure 1). Relaxed selection pressure in NDR would mitigate the natural bias towards deletion and permit genes acquired by HGT to persist in the genome, regardless of their adaptive coefficient. Microbial sectoring that accompanies geographic range expansion (70) would then allow these newly acquired genes to accumulate at intermediate frequencies in the pangenome. The fact that NDR has larger overall genome size and that relative selection pressure is lower in NDR than SDR, is contrary to the predictions of the metabolic versatility hypothesis of large genomes.

We hypothesize that relaxed selection and drift caused genome expansion in NDR. While these same mechanisms are known to promote genome reduction in endosymbionts and obligate pathogens (17, 18), it is important to recognize that these outcomes are not contradictory (Figure 6). Genome size is regulated by rates of gene gain and loss, the selective coefficient for each gene in the genome, and the strength of selection. Endosymbionts and obligate intracellular pathogens have small population sizes and accordingly, relaxed selection and stronger drift. Relaxed selection pressure should lessen deletion bias. But under these conditions, host compensation for microbial gene function radically alters selective coefficients of core genes, thereby favoring genome reduction, and slightly deleterious mutations accumulate over time via Muller's ratchet (71, 72). In addition, rates of HGT from non-host sources are essentially zero, since there is little opportunity for endosymbionts to interact with other microbial cells, resulting in a one way track to genome erosion. In contrast, for free-living microbes relaxed selection pressure should bring about genome expansion by shifting the selective coefficients of accessory genes towards neutral. For example, genome expansion in *Chlamydia* was driven by relaxed selection, recombination, and introgression (69). In this way small population size can favor genome erosion in endosymbionts, while also favoring genome expansion in free-living organisms (Figure 6). Meanwhile, free-living organisms that have large population sizes and high selection pressure will experience high rates of deletion that purge unnecessary genes in order to promote genome streamlining (14, 15).

Newly acquired genes tend to occur at low frequency in a population unless they provide an adaptive benefit (73), while adaptive genes will increase rapidly in frequency to join the core genome. These dynamics are believed to explain the characteristic U-shape of pangenome gene frequency distributions (51, 74). Deviations from U-shape expectations, including increased intermediate frequency genes, can result from changes in selection coefficients of genes or under conditions where HGT exceeds deletion rates (75). Alternatively, negative frequency dependent selection can cause highly beneficial genes to occur at low and intermediate frequencies (76, 77). A large portion of rare genes in microbial pangenomes are hypothetical proteins or genes of unknown function acquired through HGT (78, 79). For both NDR and SDR, approximately 60% of unique-rare genes (i.e., present in 1–2 strains) are annotated as hypothetical proteins. Nearly half of the 2,596 genes in NDR's intermediate-low frequency gene pool (i.e., present in 3–5 strains) are also hypothetical genes. Furthermore, intermediate-low frequency genes are similar to rare frequency genes in regards to GC content (Figure S3) and codon usage (Figure S4). These data are consistent with our hypotheses that NDR intermediate frequency genes represent evolutionarily recent HGT-gene acquisitions, which increased in frequency as a result of genome surfing.

HGT-mediated genome expansion supplies a reservoir of novel genetic material for the evolution of gene families (25, 26), biosynthetic pathways (80), and formation of new metabolic networks (81). Hence, the metabolic versatility of large genomes might be a classic example of an evolutionary spandrel (82), an adaptive trait associated with large genomes that originated not because of selection for versatility, but rather because the acquisition of diverse metabolic pathways is a byproduct of non-adaptive evolutionary process that cause genome expansion.

We show that pangenome analysis of *Streptomyces* sister-taxa verifies several predictions of the hypothesis that genome expansion within this clade was enabled by non-adaptive evolutionary processes, most likely driven by late Pleistocene demography. We hypothesize that small effective population size and relaxed selection, a consequence of geographic range expansion, allowed for genes newly acquired by HGT to increase in frequency within the NDR pangenome as a result of genome surfing. Further amplifying this effect is introgression of genes from local gene pools encountered following dispersal into new environments. Non-adaptive genome expansion is inherently a non-equilibrium process driven by a transient period of relaxed selection, and population stabilization will re-impose selection pressures that favor deletion. At this point, intermediate frequency genes will either be lost to deletion or fixed if they provide adaptive benefits, and these processes will shift the pangenome structure back to U-shaped expectations. These insights highlight the importance of considering population demography and the profound influence of historical contingency on contemporary patterns of microbial genome diversity.

Material and Methods

Streptomyces isolation and genomic DNA extraction

The strains in this study belong to a larger culture collection of *Streptomyces* isolated from surface soils (0–5 cm) spanning sites across the United States (see (45, 46)) (Table S1). To minimize the effects of environmental filtering in driving patterns of microbial diversity, we selected sample locations with similar ecologies including meadow, pasture, or native grasslands dominated by perennials and with moderately acidic to neutral soils (pH 6.0 ± 1.0, mean ± SD).

Streptomyces strains were isolated by plating air-dried soils on glycerol-arginine agar (pH 8.7) plus cycloheximide and Rose Bengal (83, 84) as previously described (60). Genomic DNA was extracted with a standard phenol/chloroform/isoamyl alcohol protocol from 72 h liquid cultures grown at 30˚C with shaking in yeast extract-malt extract medium (YEME) + 0.5% glycine (59).

Genome sequencing, assembly, and annotation

Genome sequencing, assembly, and annotation is previously described (see (47)). Briefly, we used the Nextera DNA Library Preparation Kit (Illumina, San Diego, CA, USA) to prepare sequencing libraries. Genomes were sequenced on an Illumina HiSeq2500 instrument with paired-end reads (2 x 100 bp). Genomes were assembled with the A5 pipeline (85) and annotated with RAST (86). This generated high quality draft genome assemblies with over 25X coverage and estimated completeness > 99% as assessed with CheckM (87). We used ITEP and MCL clustering (inflation value = 2.0, cutoff = 0.04, maxbit score) (88) to identify orthologous protein-coding gene clusters (i.e., genes). Genome sequences are available through NCBI under BioProject PRJNA401484 accession numbers SAMN07606143–SAMN07606166.

Phylogeny

Phylogenetic relationships were reconstructed from whole genome alignments. We used Mugsy (89) to generate multiple genome nucleotide alignments and trimAl v1.2 (90) for automatic trimming of poorly aligned regions. Maximum likelihood (ML) trees were built using the generalized time reversible nucleotide substitution model (91) with gamma distributed rate heterogeneity among sites (GTRGAMMA) in RAxML v7.3.0(92), and bootstrap support was determined following 20 ML searches with 100 inferences using the RAxML rapid bootstrapping algorithm (93). Average nucleotide identity (ANI) was calculated from whole genome nucleotide alignments using mothur (94).

Pangenome and population genetics analyses

The pangenome was determined from the gene content of 24 *Streptomyces* genomes (Table S2). Strains in this collection were initially chosen for whole genome sequencing based on their genetic similarity at house-keeping loci (see (46)). Subsequent analyses focused on recently diverged sister-taxa clades of 10 genomes each, the northern-derived (NDR) and southern-derived (SDR) lineages. Gene content patterns between strains and pangenome gene frequency distributions were determined from gene presence/absence data.

Gene-level attributes across gene pools were determined from the average of all nucleotide sequences within an orthologous protein-coding gene cluster (see above). GC content was calculated for each gene using the R package Biostrings (95). Codon usage bias was calculated for each gene using the R package cordon (96). Clade-level population genetic traits were evaluated using 2,778 single-copy genes conserved across all 24 genomes. For each core gene, nucleotide sequences were aligned using MAFFT v.7 (97), and Gblocks (98) removed poorly aligned positions. PAL2NAL (99) generated codon alignments, and SNAP (100) calculated intra-clade non-synonymous (K_A) and synonymous (K_S) substitution rates (values > 2 were filtered prior to plotting and statistical analysis).

Demographic simulation

We assumed that the SDR pangenome approximates the gene frequency distribution of the last common ancestor of NDR and SDR. For the starting generation 0, we used the model from Marttinen *et al.* (55) to simulate a population of sequences and learn parameter values for rates of gene acquisition and deletion that produced the frequency distribution for SDR. To model range expansion demographics (i.e., severe bottleneck followed by exponential growth), we sampled 5 strains from generation 0 as the founding population for the subsequent generation, and simulated this for 100 generations. The simulated population had a growth rate of 5% per generation until a maximum of 100 individuals was reached. We varied the initial sizes of the founding population as well as the growth rate, and observed qualitatively similar results.

The model included gene acquisition events and deletion events similar to Marttinen *et al.* (55) but modified to allow for multiple changes. Instead of acquisitions/deletions happening independently, there were k=20 simultaneous acquisitions/deletions per strain per generation. The previous model (55) included a multiplicative fitness penalty of 0.99 for each gene exceeding a pre-specified genome size threshold. During the expansion, we relaxed the penalty for excess genes to $0.99^{(\text{current size/max size})}$ allowing for genome size variation.

Data Availability

Streptomyces genome sequences are available through NCBI under BioProject PRJNA401484 accession numbers SAMN07606143–SAMN07606166.

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Figure Legends

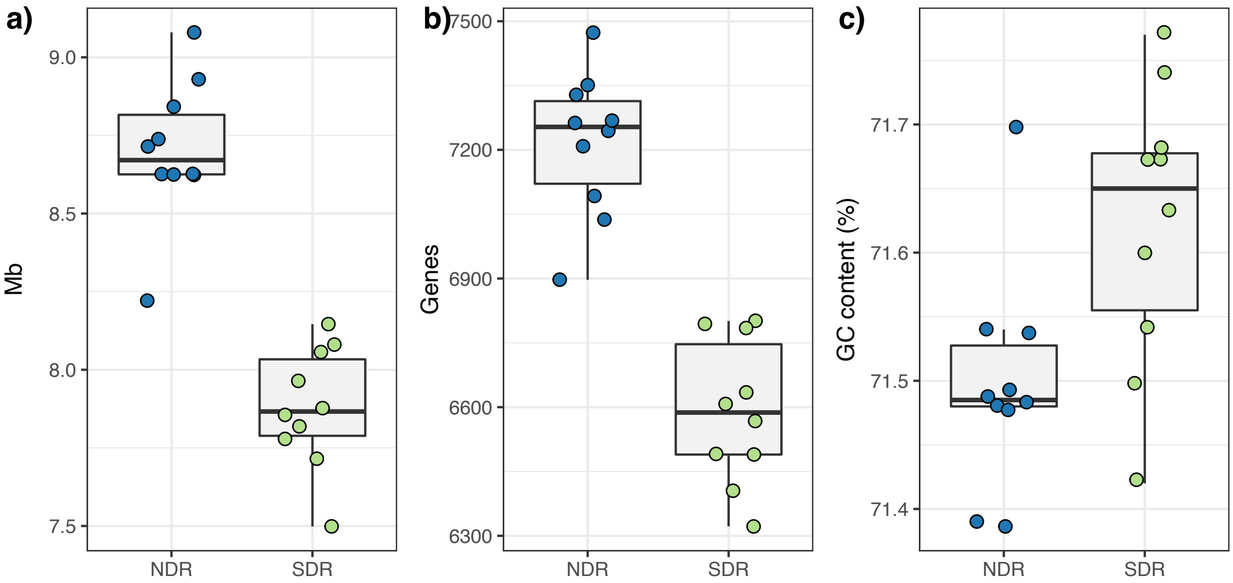
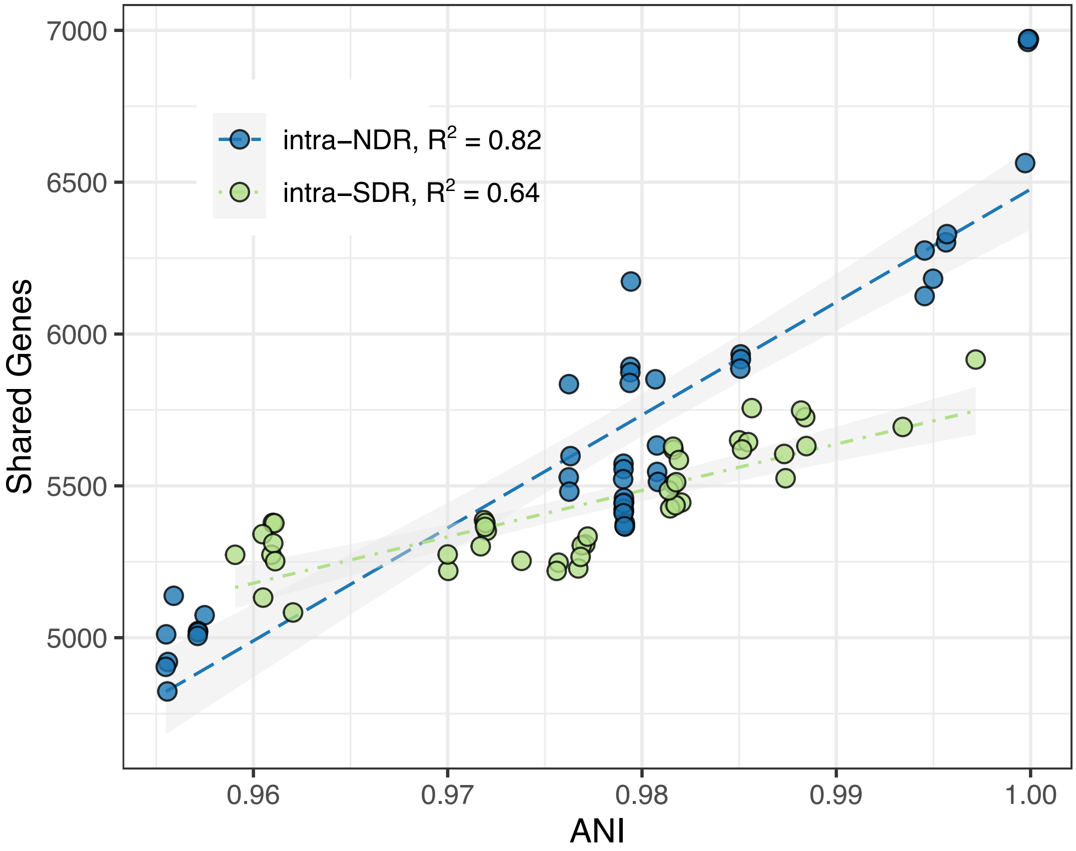


Figure 1. Genomic attributes of NDR and SDR sister-taxa. NDR genomes are larger, have more genes, and have lower GC content compared to SDR genomes. Plots show the distributions of genome size in Mb (a), number of genes (b), and genome-wide GC content (%) (c) for *Streptomyces* sister-taxa. Boxplots show the clade-level medians, interquartile ranges, and 1.5 times interquartile ranges. Colored circles illustrate the values for individual genomes belonging to the NDR clade (blue) or the SDR clade (green).



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698 **Figure 2.** Genomic similarity versus shared gene content for NDR and SDR. Differences in shared gene content across increasing average nucleotide identity (ANI) are greater within the NDR clade compared to the SDR clade (Table S3). Circles show pairwise comparisons of the number of shared genes between two strains versus ANI and are colored by clade according to the legend. Dashed lines show linear regressions, and the shaded area is the 95% confidence interval.

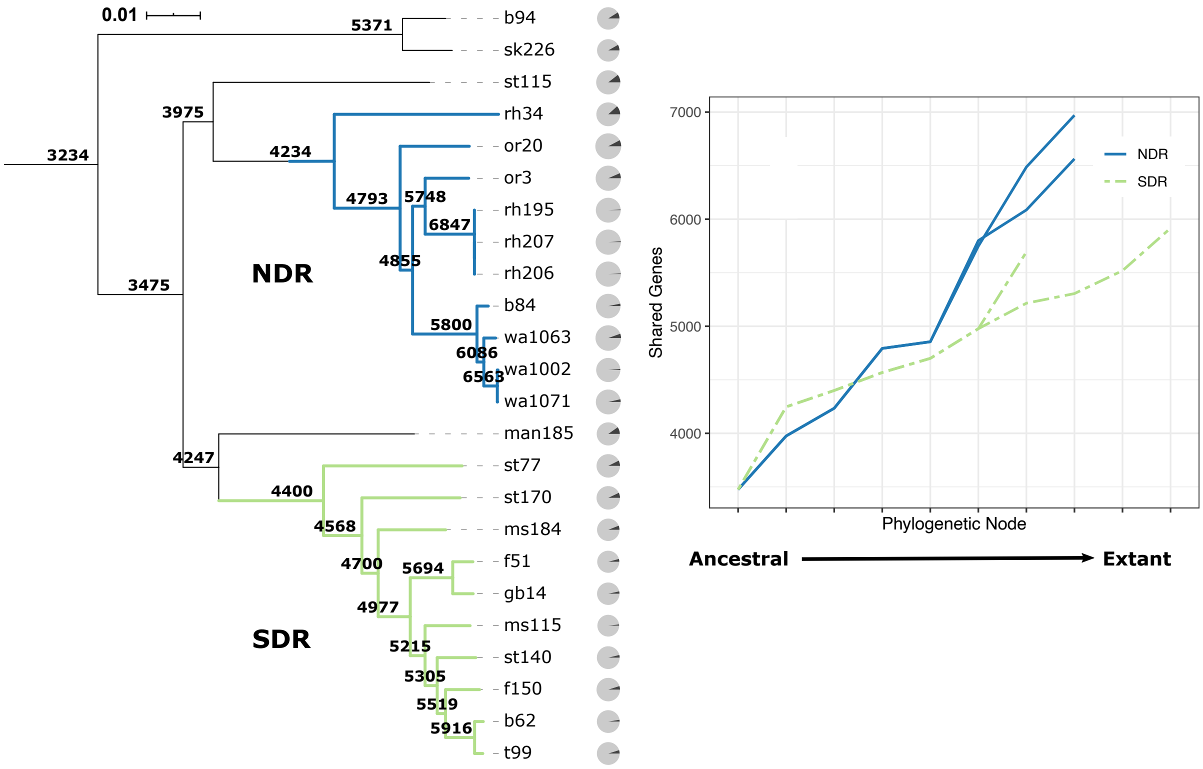
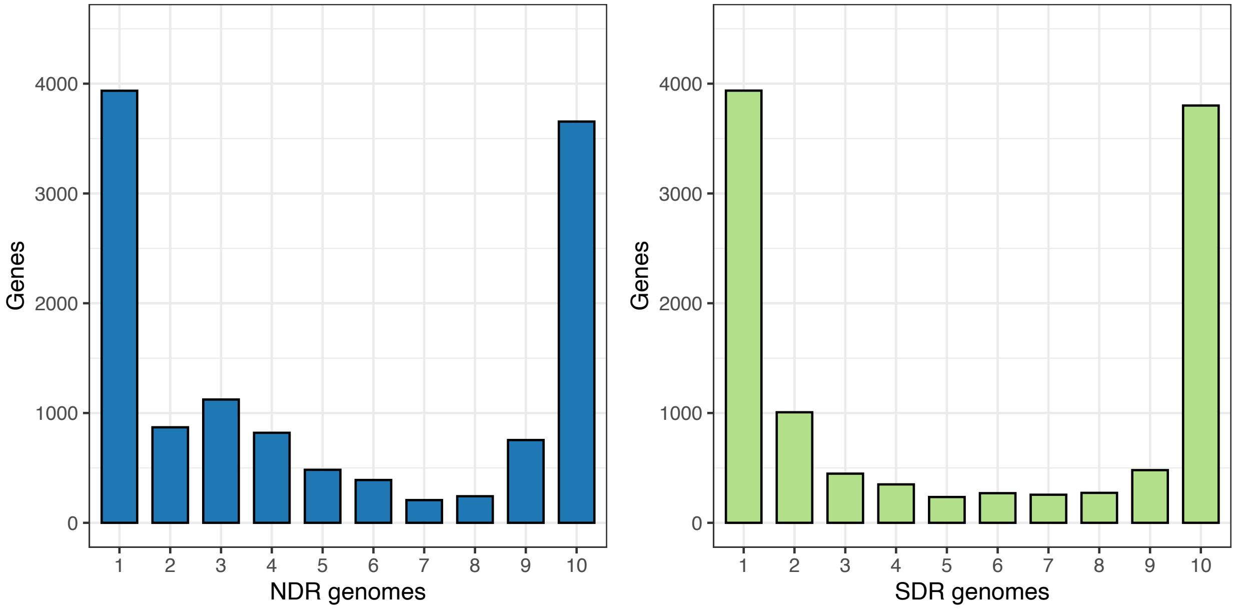


Figure 3. Presence/absence of genes across phylogeny. Gene content changes more rapidly across ancestral phylogenetic nodes for NDR genomes compared to SDR genomes. Tree is made from whole genome nucleotide alignments, and the scale bar shows nucleotide substitutions per site (see Figure S1). Branch colors reflect clade membership. Phylogenetic nodes are labeled with the number of genes conserved in all members of descendent nodes. Gray pie charts at tree tips show the portion of total genes per genome that are strain-specific (black slice). Right panel plots the differences in gene content across the phylogeny beginning at the shared ancestral node and ending with extant taxa at the terminal tips for NDR (blue-solid) and SDR (green-dashed) lineages. Multiple lines represent monophyletic lineages.



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715 **Figure 4.** Pangenome gene frequency distributions. NDR genomes are enriched in intermediate frequency genes. Plots show the pangenome gene frequency distributions for NDR (left) and SDR (right). Bars show the population-level sums of genes present in 1–10 genomes. See Table S3 for raw values and proportions.

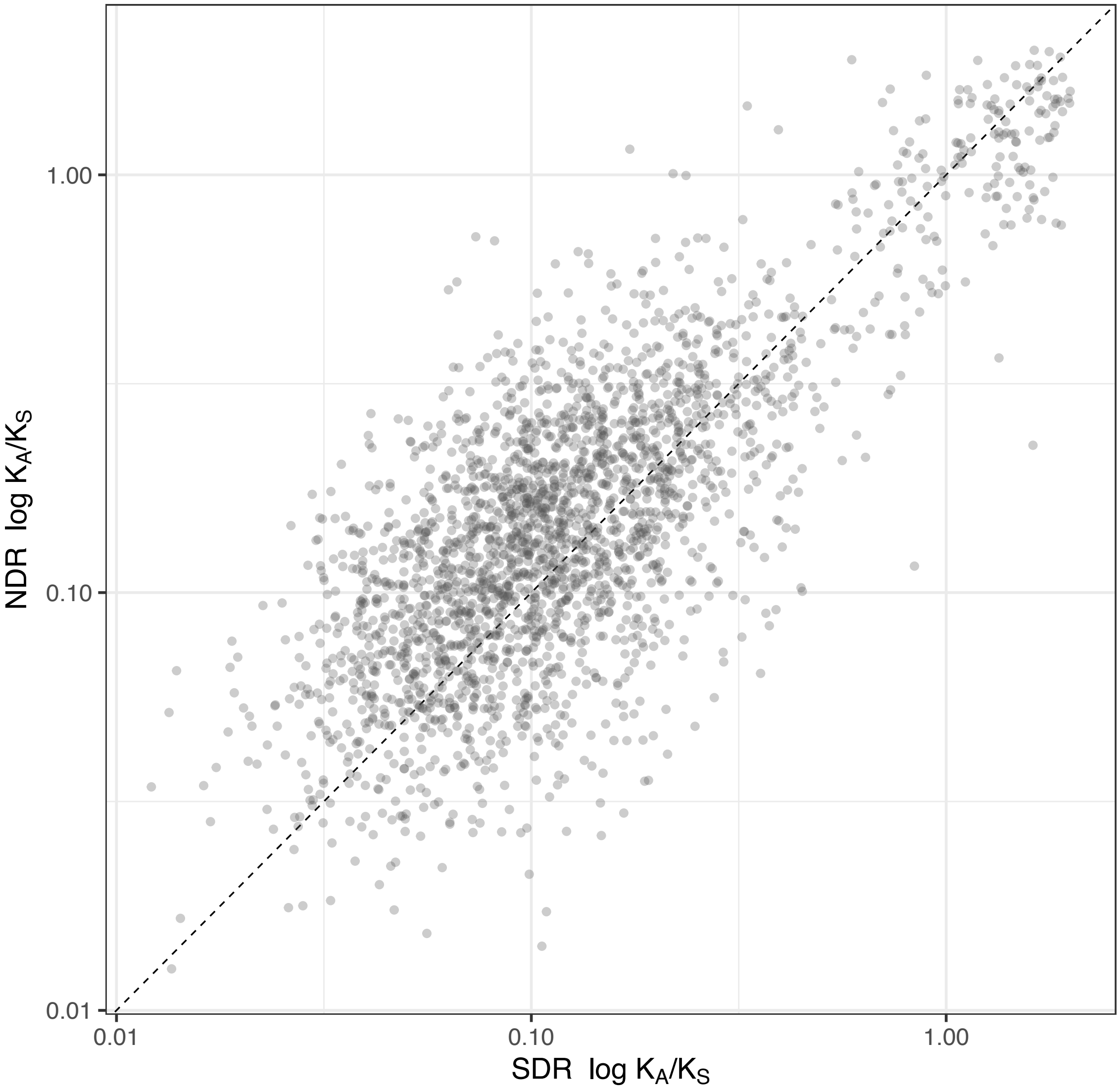

Figure 5. K_A/K_S values between the NDR and SDR sister-taxa core genome. NDR core genes have, on average, greater rates of non-synonymous to synonymous amino acid substitutions compared to SDR core genes. Circles plot clade-level rates of non-synonymous to synonymous amino acid substitutions (K_A/K_S) for each of 2,444 single-copy core genes for NDR (y-axis) and SDR (x-axis). Axes are logarithmic scale. The black dashed line is a slope of 1, and points along this line are genes with equal K_A/K_S mean values in both clades. K_A/K_S is proportional to the relative strength of genetic drift and inversely proportional to the relative strength of selection.

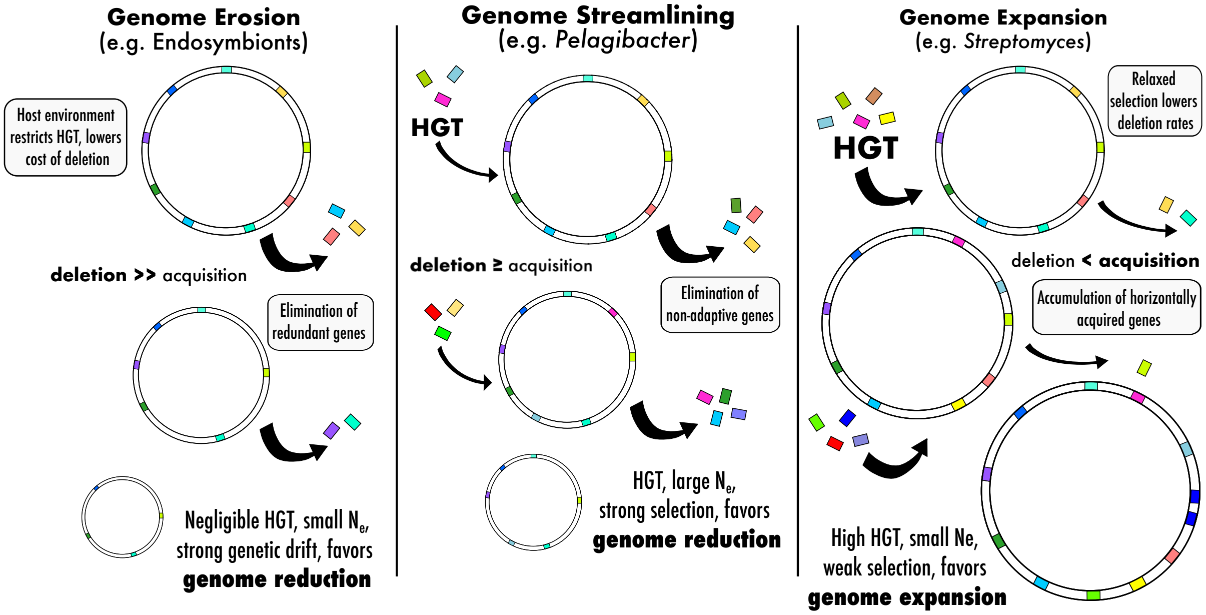
Genome Erosion
(e.g. Endosymbionts)
Host environment restricts HGT, lowers cost of deletion
deletion >> acquisition
Elimination of redundant genes
Negligible HGT, small N_e, strong genetic drift, favors genome reduction
Genome Streamlining
(e.g. Pelagibacter)
HGT
deletion ≥ acquisition
Elimination of non-adaptive genes
HGT, large N_e, strong selection, favors genome reduction
Genome Expansion
(e.g. Streptomyces)
Relaxed selection lowers deletion rates
HGT
deletion < acquisition
Accumulation of horizontally acquired genes
High HGT, small Ne, weak selection, favors genome expansion

Figure 6. Conceptual overview of the evolutionary processes and demographic conditions that support changes in genome size. Genome erosion (left) in endosymbionts is the result of small N_e and strong genetic drift, with host compensation lowering costs of deletion while restricting gene flow (HGT). Genome streamlining (middle) in free-living microbes with large populations like *Pelagibacter* involves strong selection and elimination of non-adaptive genes. Genome expansion (right) in *Streptomyces* is facilitated by high rates of HGT and relaxed selection, allowing for the accumulation of non-adaptive genes and ultimately larger genomes.

Supplemental Material

Table S1. Strain sample location and metadata.

Table S2. *Streptomyces* genomic attributes and metadata.

Table S3. Shared gene content and genomic similarity linear model summary.

Table S4. Pangenome frequency distributions.

Figure S1. Whole genome phylogeny and map of sample locations.

Figure S2. Genome-wide gene density plots.

Figure S3. Mean GC content across gene frequency pools.

Figure S4. Mean codon bias across gene frequency pools.

Figure S5. Demographic simulation.