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Prokaryotic species are *sui generis* evolutionary units

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ABSTRACT

Many gene flow barriers associated with genetic isolation during eukaryotic species divergence, are lacking in prokaryotes. In these organisms the processes associated with horizontal gene transfer (HGT) may provide both the homogenizing force needed for genetic cohesion and the genetic variation essential to speciation. This is because HGT events can broadly be grouped into genetic conversions (where endogenous genetic material are replaced with homologs acquired from external sources) and genetic introductions (where novel genetic material is acquired from external sources). HGT-based genetic conversions therefore causes homogenization, while genetic introductions drive divergence of populations upon fixation of genetic variants. The impact of HGT in different prokaryotic species may vary substantially and can range from very low levels to rampant HGT, producing chimeric groups of isolates. Combined with other evolutionary processes, these varying levels of HGT causes diversity space to be occupied by unique groups that are mostly incomparable in terms of genetic similarity, genomic cohesion and evolutionary age. As a result, the conventional, cut-off based metrics for species delineation are not adequate. Rather, a pluralistic approach to prokaryotic species recognition is required to accommodate the unique evolutionary ages and tendencies, population dynamics, and evolutionary fates of individual prokaryotic species. Following this approach, all prokaryotic species may be regarded as unique and each of their own kind (*sui generis*). Taxonomic decisions thus require evolutionary information that integrates vertical inheritances with all possible sources of genetic heterogeneity to ultimately produce robust and biologically meaningful classifications.

Introduction

In eukaryotes, speciation is often described as divergence between distinct populations that leads to reproductive incompatibility upon subsequent contact between these diverged populations [1–5]. This is fundamentally different from prokaryotes that reproduce asexually, usually through binary fission, and thus lack many of the gene flow barriers linked to eukaryotic speciation [6,7]. However, genetic isolation is still required for speciation, because mechanisms are needed to overcome promiscuous genetic exchange between species. Prokaryotic speciation thus involves restriction of the homogenizing effect of gene flow (Table 1) through horizontal means between diverged subpopulations [3,8,9].

In this review, we address some of the issues associated with studying prokaryote evolution. To accomplish this we first discuss how genetic variation as the basis for speciation is introduced in populations. We then discuss the effects of horizontal transfer of genetic material, as one of the main sources of variation, within and between populations and species. This is followed by considering the evidence for how prokaryotic diversity is structured and by proposing an integrated model that incorporates the prevailing ideas on speciation and that accommodates all prokaryotic taxa. Finally, we consider how the process of speciation in prokaryotes occurs and conclude by discussing the integrated model in light of the most prominent species concepts and examining the implications for prokaryotic systematics. Through this review, we aim to demonstrate that individual prokaryote species are uniquely evolving groups and are thus *sui generis* (unique or of its own kind) in nature.

Sources of genetic variation

Genetic variants in a population has been described by Arber [10] as the "substrate for selection . . . exerted by the living conditions encountered by the organisms". However, very few genetic changes provide a selective advantage (see Selection, Table 1; [11,12]), as most changes are deleterious or lethal [10,13]. Most

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Table 1
Concepts and terms relating to the description of prokaryotic speciation.

Term/Concept	Definition
Accessory genes/genome	Genes present in the genomes of individuals of a taxon, but that are not shared by all members or that are unique to some members [45]. These genes are thought to contribute to niche specialisation and ecology [45]. They are also thought to be more prone to HGT and can alter the biology of their prokaryotic hosts through single DNA acquisition events [28,6] (See core genome and pan genome.)
Core genes/genome	Genes present in the genomes of all individuals of a taxon [45]. These genes are usually associated with housekeeping and essential cellular processes and are thought to be transferred through vertical inheritance [45,98]. The majority of these genes can be considered informational genes, as they are involved in replication, transcription and translation, as well as genes for maintaining cellular integrity and functioning (cell wall, respiration, basic metabolism) [98,107]. (See accessory genome and pan genome.)
Drift	A random process that leads to changes in allele frequency, particularly in small populations, through the fixation of certain alleles in a population, as extinction of other alleles occur [70,71]. This process can limit genetic variation within a population [20,70].
Epistasis	Epistasis is generally defined as the interaction of genes with one another [104–106]. Mutations that change epistatic interactions among genes can either be beneficial (positive) or detrimental (negative) [106]. The main type of epistatic interaction relevant to prokaryotic evolution is negative epistasis, characterized by genic incompatibilities [7]. The process is particularly prevalent during divergence of populations, when interacting loci undergo compensatory and/or complementary changes to allow continued interactions within a population [2]. Such independently fixed mutations between the two diverging lineages will thus not be compatible upon reintroduction of the alleles into an individual [2,107].
Gene/genetic conversions and introductions	Conversions result from the replacement of genomic regions (that may contain genes) with homologous DNA [36,37]. The process is homology-dependent and can occur through the integration of a copy from an outside source [36,38] or through intragenomic replacement where multiple copies within the genome are found [36,38]. Conversions involving HGT-associated replacement of native DNA with homologous DNAs from lateral sources may occur among members of a species and ultimately lead to intraspecies homogenization. Introductions result from the acquisition of novel DNA within the genome of a prokaryotic individual. This DNA has no homology to genetic material previously present within the genome of this individual. The process of acquiring such novel DNAs have been referred to as heterologous recombination [13]. This process thus allows the acquisition of novel traits and can alter the evolutionary trajectories of the individuals potentially resulting in divergence if homogenizing conversions are absent or limited.
Gene flow	The transfer of genes from one individual to another through lateral means (i.e., horizontal gene transfer [HGT]) [18,19]. This includes intraspecific, as well as, interspecific transfer of genetic material and indicates a sharing of gene pools [18,28,31,45]. Gene flow can be mediated through MGEs (See mobile genetic elements) or can occur through natural transformation [8,13,18,19,28,29,31,97]. After the introduction of DNA, genetic material can be integrated randomly into the genome [13] or through homology-dependent mechanisms [8]. (See homologous recombination.)
Homologous recombination	A process that replaces DNA with homologous DNA from endogenous or exogenous sources [39]. This process is thought to allow DNA repair through the replacement of potentially detrimental copies of an allele [39,100] and to introduce genetic variation through recombination with endogenous or exogenous homologous DNA [39]. The process involves the invasion of the native double-stranded DNA with a “new” single-stranded DNA and the subsequent replacement of one of the native strands with the new homologous DNA [39]. Recombinant joints are then resolved using processes linked to normal cellular replication [39].
Mobile genetic elements (MGEs)	All nucleic acid segments that can facilitate their own movement within or between genomes [25]. These include transposable elements, plasmids and viruses [25]. All MGEs can facilitate the transfer of their own genetic material as well as potentially transferring other DNA from the donor cell [19,25]. MGEs may represent HGT mediators, as conjugative plasmids or integrated elements can facilitate conjugation [19,25], while viruses are responsible for transduction [25]. These elements can be involved in both genetic introductions and conversions, through HGT events or genomic shuffling [25]. Recombination between various types of MGEs can occur quite easily, allowing the introduction of more variation within MGEs with strict host ranges [29,55,56,62], thus contributing toward the variation that can be introduced into novel prokaryotic host cells.
Mutation	Genetic changes occurring at local and global scales within the genome [3,10,14,15,17]. These changes can be caused by transitions, transversions, insertions (endogenous or exogenous source) and deletions [10], resulting in point mutations or global genomic changes [10]. Mutations can be introduced by endogenous processes like site-specific recombination systems [10], but can also result from external causes like MGEs or environmental factors [10].
Pan genome	All genes present in at least one individual within a species, often referred to as the species genome [45]. The pan genome consists of the accessory and the core genomes [45]. Some species are thought to have “open pan genomes” with large accessory genomes where many additional genes are identified as more genome sequences for the species become available. By contrast, others have “closed pan genomes” where the majority of the genomes are relatively homogenous in terms of sequence and gene content (thus sequencing additional genomes do not add many genes to the pan genome) [60,61,81]. (See accessory genome and core genome.)
Plasmid incompatibility group	Plasmid incompatibility is generally described as the lack of stable inheritance of plasmids upon introduction of an additional, different plasmid into a host cell [63]. The second plasmid destabilizes the inheritance of the first and is thus considered as incompatible [63,64]. The main mechanism of incompatibility is attributed to the same origins of replication within different plasmids, as these plasmids will compete for replication factors [63,64].
Selection	Selection occurs through the increased survival and reproduction of specific phenotypes (at the molecular, morphological or physiological level) [11]. This process allows environmental forces to select for individuals with increased fitness [11]. Over time, this process can lead to the homogenization of populations through selective sweeps, eliminating less fit individuals or loci within the population [8,13,42]. Two types of selection are particularly relevant to the discussion of prokaryote evolution, namely second order selection and divergent selection. Second order selection can occur where no selectable gene products are produced [10,12]. This type of selection is usually associated with cellular systems where the effect of a particular system can be selected for, as the systems itself cannot be directly selected for [12], e.g. systems modulating the introduction of variation and variation generators. Divergent selection is described as selection favouring divergence of individuals to attain increased fitness [82]. In this scenario, individuals within a population shows increased evolvability by maintaining higher levels of variation within the population through selective pressures [3,31,82].

Table 1 (*Continued*)

Term/Concept	Definition
Sexual recombination	Sexual recombination in eukaryotes can be described as recombination between two nuclei originating from different meiotic events (karyogamy; [33–35]). This process results in the recombination of genetic material from two parental organisms [8,3-35]. The process is also linked to reproduction [8,35].
Site-specific recombination	Contrary to homologous recombination, extensive homology is not required for recombination to occur, although specific recognition sites (usually showing short regions of homology) are required for crossovers to occur [22–24]. The process typically involves breaking of double-stranded DNA in a staggered manner at the recognition sites, followed by joining of the broken DNA molecules through covalent binding by a recombinase enzyme [22–24]. This system can also be employed for DNA transposition [23].
Species boundary	Often described as the borderline between population divergence and population level tokogenetic processes [7,114,129–132]. Although not a physical impenetrable barrier the species boundary can be interpreted as regions in diversity space enveloping cohesive groups, which resulted from barrier traits maintaining separation between groups [7,9]. The species boundary thus needs to be determined from an evolutionary perspective and not using predetermined cut-off values, as different cohesive groups are not equivalent [28,95,114].

spontaneous genetic variation is not a product of the organism's need, but rather a product of stochastic processes [10], followed by purging or fixation of variants within a population. These genetic variations can broadly be attributed to three main mechanisms (see Mutation, Table 1) [10,14–16]. They include small mutational changes like point mutations (transitions, transversions, insertions, deletions) and small-scale nucleotide reshuffling [3,10], genomic shuffling where genome segments are duplicated, excised and/or moved, to different parts of the genome [10,14], and horizontal gene transfer (HGT) [3,14,15,17] during which genetic material may be acquired through means other than vertical descent [18,19]. These three mechanisms do not contribute equally toward speciation and the frequency of their occurrence can differ among populations [10].

The processes responsible for introducing these various types of genetic changes can either be stochastic or specific [10]. Stochastic variation is introduced through random mutations being fixed within the genome and often results in small mutational changes [20]. On the other hand, specific or dedicated systems contributing toward variation can either act as variation generators, or as regulators of the frequency at which variation is introduced (i.e., variation modulators). Examples of systems responsible for generating variation are site-specific recombination systems (Table 1; [10,21–24]) and mobile genetic elements (MGEs, Table 1; [10,25]). In contrast, systems that regulate the frequency of the introduction of variation are DNA repair, restriction modification systems [10,26] and potentially also the CRISPR-Cas system [27]. In relation to the sources of variation, the DNA repair and site-specific recombination systems contribute toward controlling the variation introduced through small mutational changes and genomic shuffling, while MGEs and restriction modification systems largely control the variation introduced through HGT [10].

HGT as a driver of evolution

In light of the asexual nature of prokaryotes, the genetic diversity observed in these organisms is too high to have mainly been caused by endogenous processes [17,28,29]. Thus, prokaryotic evolution can only be described by vertical inheritance together with horizontal acquisition of genetic material [29,30]. HGT can also be considered as an integral part of evolution in these groups [3,8,13,17,19,29,31,32], due to the immense adaptive potential it provides [6,29]. Contrary to genetic exchange through sexual recombination (Table 1; [8,33–35]) in eukaryotes, the transfer of genetic material among prokaryotes is usually unidirectional and can be facilitated through mediators like MGEs [8,29,35]. This process allows the exchange of genetic information among members of the same species or among very distantly related species and can involve genetic introductions and conversions (Table 1) or combinations of these (see Fig. 1; [3,8,13,14,28,29,36–39]).

Genetic conversions linked to HGT may represent a strong homogenizing force within populations, because they can occur among members of the same species (Fig. 2a; [3,40]). This HGT-mediated process thus involves selective sweeps occurring at both the organismal [8,13,41,42] and genic [8,40] levels. The homogenizing effects of HGT within populations have been elegantly demonstrated in *Shewanella baltica* and *Streptococcus pneumoniae*. In *S. baltica* populations in the Baltic Sea, genome-wide genetic conversions have been shown to routinely purge variation from the population faster than point-mutations are introduced [40,43]. These *S. baltica* populations serve as an example of cohesion resulting from genic level selective sweeps across the genome. In *S. pneumoniae* [44], naturally competent individuals have been shown to mediate the lysing of non-competent cells as a means of obtaining more, potentially useful, free DNA in the environment and also to out-compete non-competent individuals within the population [3,44]. In this instance, organismal level selective sweeps maintain the cohesion amongst individuals, because less fit (non-competent) individuals are purged from the population.

In contrast to intraspecific HGT, genetic introductions and conversions between members of different species can aid divergence (Fig. 2b; [15,35]). Because HGT is often not limited to single genes and can involve large genomic regions, containing multiple genes [45,46], their acquisition can alter the biology of the individuals harbouring them. This is particularly true in terms of niche-associated characters where genetic introductions can produce recombinant individuals with the ability to survive and thrive in niches different from those of the non-recombinant parent [47,48]. In these situations, the spatial separation (due to niche differentiation) between these subpopulations, originating from these two individuals, allows genetic isolation and would thus drive the distinct evolutionary trajectories of the two groups [48]. An example of where this may be the case is *Salmonella* Typhi, where it is thought that the lack in purging of diversity within the group is linked to the fact that at least two diverged subpopulations exist, occupying distinct niches that prevents homogenization of these subpopulations [49]. One subpopulation is adapted to clinical treatment in humans with nalidixic acid as opposed to those not treated with the drug [50], allowing niche differentiation to drive divergence [49].

It is currently thought that there is no limit to which genes can be affected by HGT. It has been detected in genes that are ecologically important (niche-associated; often referred to as accessory genes, Table 1), as well as in those with housekeeping functions (often referred to as core genes, Table 1) [3,29,51]. Thus, despite the increased frequency at which niche-associated genes are transferred [3], some genes involved in transcriptional and translational processes have also been identified as horizontally acquired [29,51,52]. In fact, Dagan et al. [51], demonstrated that up to 81% of the prokaryotic genome has been subjected to HGT at some point in time. In these cases, recombination rates, or as it

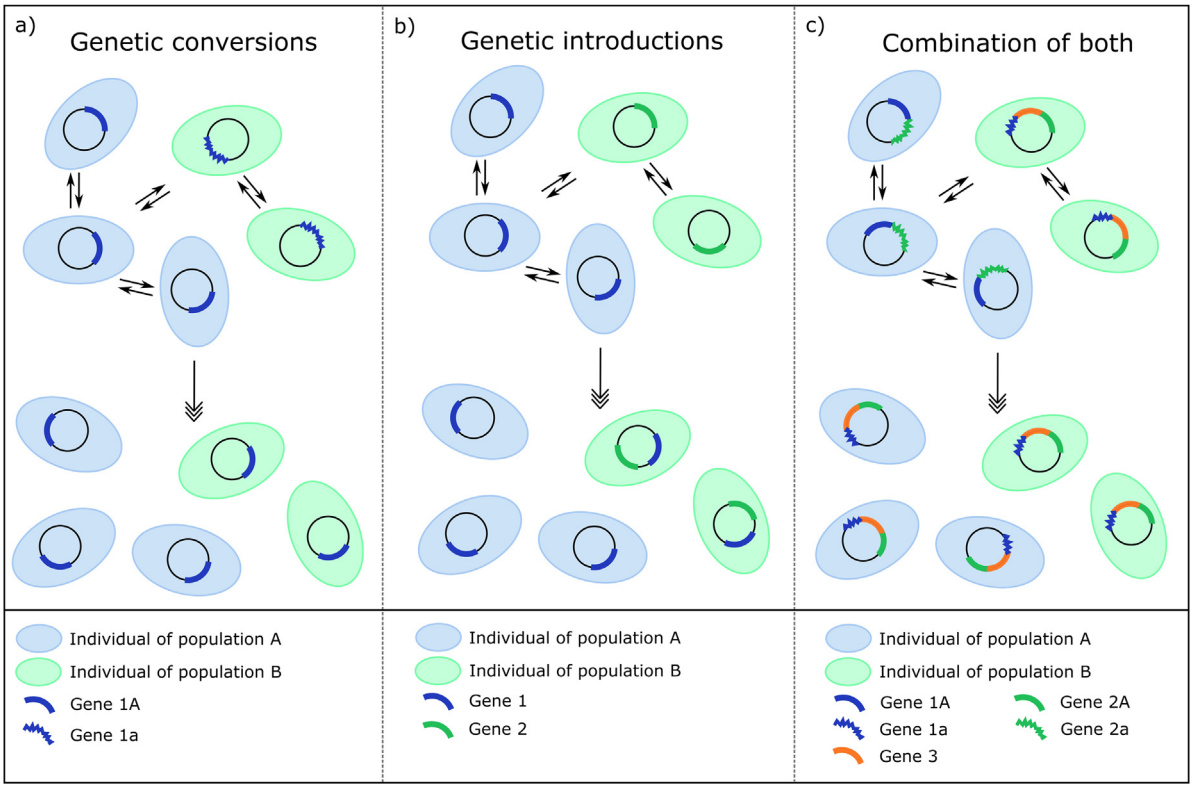

Fig. 1. Different types of HGT that occur naturally in prokaryotic populations. a) Genetic conversions are characterised by the replacement of endogenous DNA with homologous DNA from an internal or external source, e.g. replacement of one allele by another allele within or between populations (allele 1A replacing allele 1a in the host genome). Integration of DNA in the recipient genome is dependent on homologous recombination. These conversions can occur in genomic regions associated with housekeeping or accessory functions. b) Genetic introductions involves the acquisition of novel DNA that has no homology to native DNA within the host genome. Integration of novel DNA can occur randomly, which usually involves the introduction of new or accessory functions through the acquisition of genes, gene clusters or genetic islands. c) A combination of both these processes can occur in concert, where homologous allelic replacement can lead to the integration of associated novel genetic information.

were, rates at which HGT occurs, were much higher than mutation rates, with these HGT events primarily being adaptive [3,13].

Our traditional view of prokaryotic evolution, which focusses on the transfer of genetic material from parent to progeny in a vertical fashion (i.e. stable inheritance depicted by bifurcating phylogenies), is enormously affected by the presence of HGT. This is because HGT disrupts the genomic cohesion associated with vertical inheritance of cohesive genetic units. This means that what is generally considered as a sign of species boundaries (Table 1), i.e. genomic cohesion, is absent from taxa prone to HGT. This has led to many arguments against the use of phylogenies to depict prokaryotic evolution. These arguments are further supported by the fact that enforcing a bifurcating topology on non-treelike data produces phylogenetic trees, despite the lack of inheritance of traits from a common ancestor [28,29,53]. It is also thought that taxa undergoing extensive interspecific HGT would be grouped as sister to one another because of similarities in the horizontally transferred DNAs, despite the lack in vertical descent from the same ancestor [29]. Furthermore, for a bifurcating evolutionary hypothesis to be informative at all, diverged descendants need to become genetically isolated before subsequent bifurcations occur [8]. Where further divergence occurred prior to genetic isolation, no ancestral population (a bifurcating node in a phylogenetic tree) can be traced or even existed, providing false depictions of the evolutionary history [8].

The presence of continual gene flow among species is thought to give rise to organisms that are genetic chimeras [29,54]. These chimeric individuals can be argued to belong to more than one species simultaneously depending on what part of the genome is analysed [6,8,29,32,42]. This is due to the ability of these chimeras to undergo homologous recombination (genetic conversions; see Table 1 and Fig. 1a) with multiple species (all species serving as sources of horizontally acquired DNA) [6]. This idea can be developed even further, by looking at different genomic regions. As different genomic regions evolve at different rates and are subject to different evolutionary forces, some loci within the genome can act like diverged subpopulations (undergoing limited to no gene conversions), while other loci may still be recombining frequently through homology-dependent HGT [8,32]. Thus, complex genetic chimeras can exist due to 1) their genetic connectedness (via HGT-based conversions) with multiple species and 2) the different forces and processes driving evolution in certain parts of the genome, with both of these factors leading to phylogenetic incongruence between different parts of the genome.

Biological organization — genetic continuum or discrete species?

Arguments for the existence of a prokaryotic genetic continuum are largely focussed around the effects of HGT on populations and species. Hypothetically, if HGT allows an organism to consist of parts of various species simultaneously, the organism would be an intermediate between two (or more) species and would be situated within the region usually considered to be the species boundary on a genetic continuum. The main reason argued for not having observed such a genetic continuum yet is sampling inadequacies [31,32]. When we consider a spectrum of genetic diversity, sampling at specific points on the spectrum would lead to the identification of groups that appear genomically and even ecologically cohesive. Increased sampling at these points would merely provide

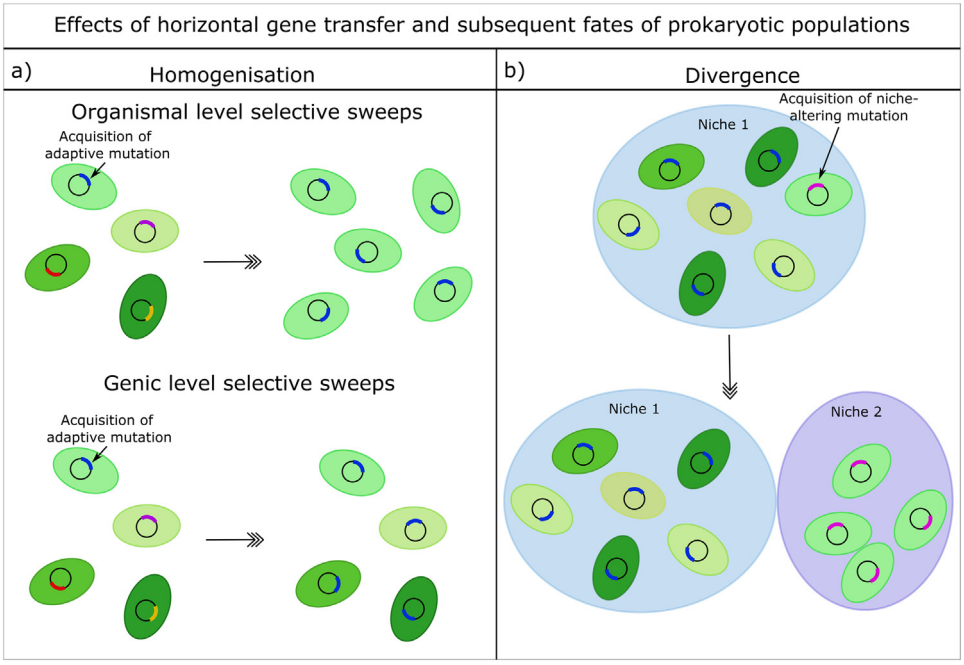

Fig. 2. Effects of HGT of adaptive mutations in naturally occurring prokaryotic populations. a) Acquisition of adaptive mutations may lead to homogenization of the population through either organism-level or genic-level selective sweeps. At the organism-level, homogenization occurs through the out-competition of less-fit individuals lacking the acquired trait. All members of subsequent generations would thus be descended from the adapted individuals. Through this process fixation of the mutation within the population occurs via the elimination of less-fit individuals, and causes within-population genetic cohesion or homogeneity. However, if intra-population gene flow occurs at sufficiently high rates, homogenization can be achieved when the adaptive locus sweeps through the population. During such a genic-level selective sweep, the adaptive locus is transferred to existing individuals with the aid of MGEs and may also result in fixation of the locus within the population (i.e., all recipients of the locus and their descendants harbour the mutation). b) If the adaptive mutation is associated with niche alteration, it may lead to divergence within an existing population (provided that movement to a new niche occurs before selective forces bring about homogenization of the population). The capacity to invade a new niche provides spatial separation between individuals with and without the acquired mutation, thus affording circumstances under which allopatric speciation (see Table 2) can occur. Depending on the frequency of HGT, this divergence can result in populations of genetic mixtures of parental populations and chimeras, often occupying the same or overlapping niches.

more isolates of the same cohesive groups and would not necessarily provide a broader view of the existing diversity. These perceived gaps in diversity could then be wrongly interpreted as species boundaries, as the intermediate organisms occurring between supposed species boundaries have not yet been sampled. Therefore, obtaining more diversity across the potential continuum is essential to determine the degree to which this confluence of genetic variation may occur in individuals [32].

In the section below, we discuss current evidence for the existence of a genetic continuum of diversity and discrete genetic clusters. We use information from both species-specific and metagenomic studies. These form the basis for the proposed integrated model of how genetic diversity in prokaryotes is structured (see below).

i) Evidence for a continuum:

Most of the studies thought to contribute evidence in support of the existence of a genetic continuum (i.e. the absence of cohesive species in nature) focuses on HGT, the chimeric individuals it produces and HGT mediators [55,56]. These studies are mostly based on genes affecting the biology of an organism, i.e., niche or host specifying genes that are often encoded on the accessory genomic compartment [6,56,57]. Indeed, any two genomes of a species can differ in up to 40% of their genes (the rest representing the core) [58,59], although these gene content differences are typically much lower. However, when investigating the complete pan genome (Table 1; [60,61]) of some species, it is possible that any particular isolate only contain ca. 5% of the potential genetic material associated with the species [6]. The majority of these non-shared accessory genes contribute to the diversity or ecological adaptations and phenotypic capabilities of the species. For some catabolic pathways, homologous gene clusters with high similarity were identified on plasmids from different incompatibility groups (Table 1) [62–64], providing some support for the independent reshuffling and genetic exchange among HGT mediators [55,56,62]. Additionally, the construction of novel catabolic pathways was also demonstrated, where the recruitment of genes from multiple genetic sources resulted in functionally novel pathways encoded for by chimeric gene clusters that were not present in parental organisms [56]. In these instances, survival, proliferation and functioning of chimeric individuals within a suitable niche presumably resulted in the establishment of long-lived populations that are intermediate between the gene donors or parental populations.

ii) Evidence for discrete species:

In contrast to the idea of a genetic continuum, data have shown that discrete groups of diversity, organised in genetically and ecologically discernible populations, exist within specific niches [28,40,65–67]. Although an apparent lack in genetic cohesion is sometimes noted when strains obtained from a variety of environments are examined (suggestive of a continuum), discrete groupings of isolates usually emerge when populations are analysed [13,40,48,68]. This is supported by evidence of extensive genetic exchange in the environment [69], but with a clear bias toward genetic conversions occurring within “sequence-discrete” (as termed by Caro-Quintero et al. [40] and that are comparable to putative species) groups or ecologically adaptive genetic introductions between non-conspecific individuals occupying the same niche [69].

The bias toward intraspecific genetic conversions and interspecies transfer of adaptive genes is also sometimes observed in interspecies comparisons. Analyses of *Campylobacter* species showed that, despite previous high estimates of genetic exchange between species, genetic conversions of housekeeping genes were

exceedingly rare [69]. Additionally, it was also seen that intraspecific gene flow occurred much more frequently than interspecific gene flow [69]. Thus, despite the transfer of ecology-related genetic information, the species' core genomes still remain cohesive and distinct between species due to the homogenizing force of frequent intraspecific genetic conversions as opposed to interspecies gene exchange.

The cohesion and distinctness of potential species clusters have also been documented in various metagenomic studies, where support for genomically cohesive clusters or populations within a community have been observed [40,65,66]. In a study by Caro-Quintero and Konstantinidis [40], whole genome shotgun sequences were obtained for a number of individuals within a community where nucleotide similarity values among genomically cohesive individuals were >90%, while those among non-conspecifics were <80%. Considering the genomic differences of these cohesive clusters at the population level, their gene content differences were often also much less (<5%) than the 35% intraspecies variation observed in *Escherichia coli* as it is currently circumscribed [40]. Thus, ecologically homogenous groups show much less genetic variation than what is often considered species, although sufficient genetic variation is still maintained for adaptational purposes. This low level of variation is also consistent with what one would expect for limited interspecies genetic exchange together with genetic drift (Table 1; [20,70,71]) over thousands of generations, providing some evidence for the longevity of sequence-discrete clusters [40]. Additionally, and perhaps most importantly, the results from these previous studies also showed that the genomic similarities at which putative species clusters form within particular environments were not necessarily the same across all taxa [3,40,72].

iii) Evidence for a combination of both:

The notions of genomically cohesive species clusters and a genetic continuum of diversity are not mutually exclusive. From the metagenomics data presented by Caro-Quintero and Konstantinidis [40], "gaps" in sequence similarities were observed among highly similar individuals (i.e., potential conspecifics) and those that were distinctly different. However, these communities also harboured "intermediate individuals", although these represented a minute proportion of the community [40]. Such intermediates within a "fuzzy" boundary zone have also been observed from the sequenced genomes available from public repositories [73]. These intermediate isolates may be evidence for individuals occurring between species boundaries on the genetic spectrum of prokaryotic diversity, proverbially "filling in the gaps". Yet, their scarcity [32,40] suggests that they either may not be fit enough to compete with other species clusters to form genetically cohesive groups of their own (i.e., they are relatively short-lived) or they may be ecologically differentiated and persist in the environment at low levels until suitable conditions favour their proliferation [54,74].

Evidence supporting a continuum of diversity together with the existence of genomically cohesive and ecologically successful clusters can also be obtained from other types of data. Genetic conversions of some housekeeping genes were detected for *Campylobacter* species and was attributed to species convergence of the closely related species *Campylobacter coli* and *Campylobacter jejuni* [75]. However, it was also shown that where the isolates of these species possessed genes from the closely related species, the isolates affected often belonged to a single phylogenetically coherent group [69,75], potentially reflective of the sequence-discrete groups found in metagenomic studies [40]. This is similar to what has been noted before for ecologically diverging species [31], as chimeric isolates may initially appear phylogenetically nested within one of the parental populations. Yet, over time, these individuals may not be able to diverge sufficiently to form their own discrete and exclusive monophyletic lineages due to their lack in success when competing with the parental populations within the community.

One could thus argue that many of the extant intermediates observed between species boundaries in diversity space (all potential genetic diversity for prokaryotes) would generally lack the potential to become successful, long-lived populations forming sequence-discrete clusters that may be described as species. Some of these clusters or species also may be genetically connected to those of other such clusters via HGT, thereby contributing toward a semi-continuous pattern of sequence similarity. With our improved ability to investigate diversity we are starting to see a genetic continuum, with variable levels of genomic cohesion occurring within different clusters from different environments. In other words, despite the appearance of a diversity continuum, genomically and ecologically cohesive clusters exist within this continuum and reflect natural groups that can be described as species.

An integrated model for prokaryote speciation

In light of the preceding arguments, we summarise the prevailing ideas on prokaryotic speciation with the aid of a model for how prokaryotic diversity is structured that integrates the requirements for both HGT and vertical descent (see Fig. 3). It also accommodates the possibility that vertical inheritance in some taxa may play a lesser role in evolution compared to HGT. The latter is often associated with niche-specifying traits, and in such taxa the effects of vertical descent may not be highly informative for predictive and taxonomic purposes [6]. This integrated model thus recognizes the intrinsic *sui generis* nature of species, by accounting for the variable forces driving their evolution.

In this model, species are considered as cohesive groups that are separately evolving from other such groups. These cohesive clusters proliferate through vertical descent and have the ability to originate and evolve through stochastic or specific evolutionary processes, operating endogenously or exogenously [10]. In terms of diversity space, all taxa do not remain cohesive at the same level. Some taxa may form tighter clusters, indicative of higher similarity levels amongst individuals such as in monomorphic species, while others may remain cohesive as looser clusters [76]. Also, there may be differences in the frequencies of HGT occurring in different cohesive clusters, with some species appearing highly resistant to the introduction of genetic material from external sources, while others frequently undergo HGT [77,78].

HGT may be associated with multiple outcomes in the integrated model. Firstly, HGT may produce individuals that are either short-lived and cannot compete within a particular niche to proliferate and establish long-lived populations or that persist in the environment until favourable conditions allow it to proliferate. Secondly, HGT may result in a clonal, monomorphic population derived from a chimeric individual, able to survive and proliferate within a suitable niche. Thirdly, multiple similar chimeric individuals may originate through independent HGT events that enable survival and proliferation of chimeras within a suitable niche, where genetic cohesion may ultimately be attained through increased HGT occurring within the cluster.

The integrated model predicts that prokaryotic species represent groups of individuals that are closer related to each other, based on genomic and phylogenetic cohesion, than to other such groups. Because they are products of evolution, they cannot be considered as equivalent groups [79–81]. This is reminiscent of the description of species by Wiley (1978) as separately evolving groups being subjected to different population dynamics that alter their evolutionary trajectories and evolutionary fates. Overall, a continuum of genetic diversity for prokaryotes may exist with many short-lived chimeric individuals, together

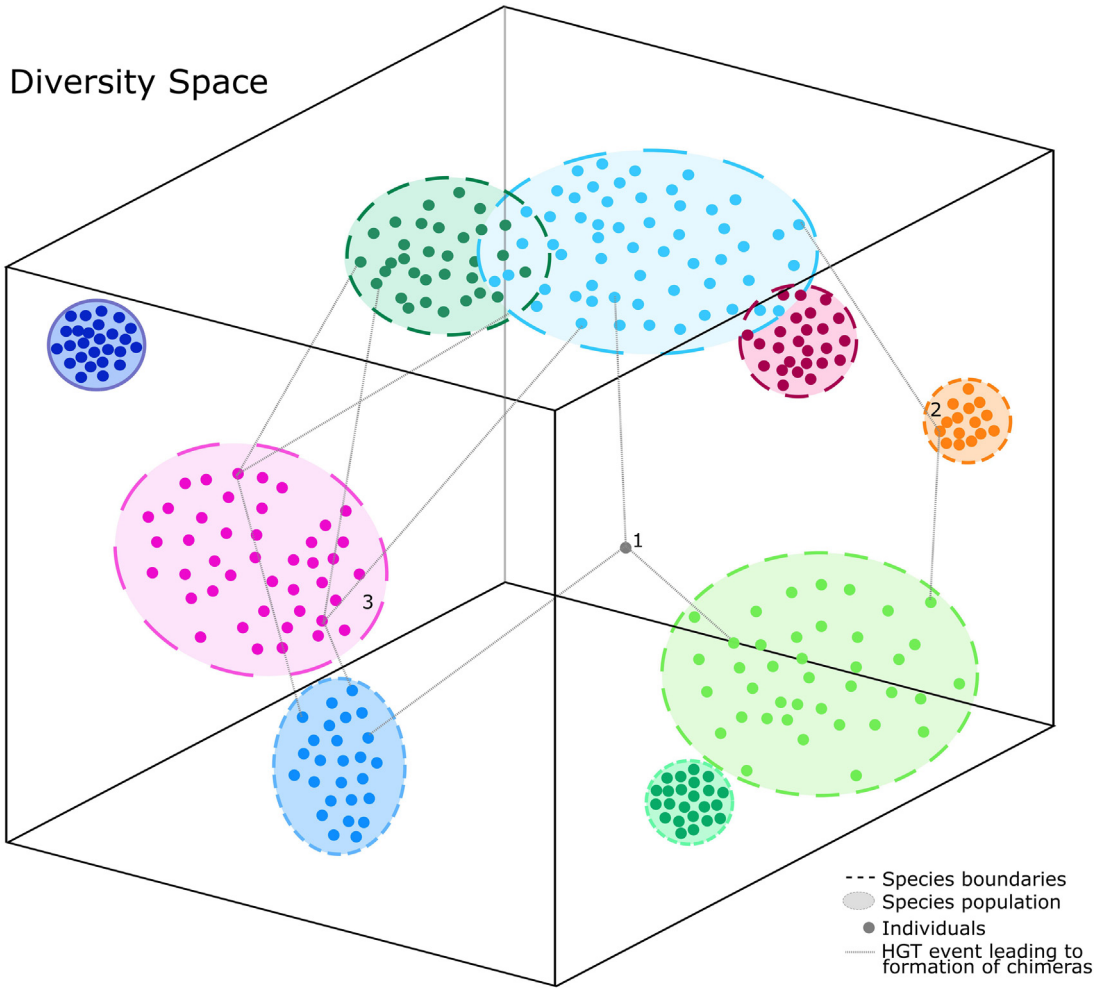

Fig. 3. An integrated model of how diversity is structured, which incorporates prevailing ideas regarding prokaryotic speciation. Dots of the same colour are representative of conspecific individuals. Dashed lines around groups of dots indicates species boundaries. The size of gaps in this boundary is indicative of the frequency of interspecific horizontal gene transfer (HGT; larger gaps represent taxa undergoing more frequent HGT than those with smaller gaps). Closely clustered groups of dots represent species in which individuals are genetically highly similar, as would be observed for clonal, monomorphic species, whereas species spanning a larger area of diversity space are more divergent, typical of species with large and divergent accessory functions. Overlapping groups represent species with ecological or genomic similarities, yet distinct evolutionary trajectories are observed, despite similarities. Chimerism due to HGT can result in 1) short-lived individuals that die out or intermediates that persist at low levels within the environment that cannot compete with native populations within a niche, 2) clonal, monomorphic populations derived from chimeric individuals or persistent intermediates originating through HGT events that could compete within their respective niches or encountered favourable environmental conditions, allowing them to proliferate and form cohesive clusters, or 3) a more diverse species group consisting of individuals originating from multiple independent HGT events resulting in multiple chimeric individuals able to survive and proliferate within a suitable niche, where continuous intraspecific HGT ultimately results in cohesion of the cluster.

with cohesive groups located at various positions in diversity space.

Speciation in prokaryotes

Under the integrated model of how prokaryotic diversity is structured, the word "speciation" refers to the process leading to the formation of genomically cohesive clusters of individuals that evolve together. The main contributors toward divergence or population differentiation are geographic or ecological isolation, genetic isolation (i.e., prevention of homogenizing gene flow through HGT-based conversions) and subsequent divergent selection (Table 1) [3,31,82]. Where differentiation between subpopulations has occurred to the level that homogenizing gene flow cannot lead to convergence of taxa, the process of speciation has concluded. The likelihood of speciation occurring thus depends on the balance between lineage splitting (divergence) and lineage mixing (reticulation) [9]. In all instances, initial divergence, due to ecological or genetic incompatibilities (see Epistasis in Table 1) can be subjected to divergent selection leading to further differentiation between subpopulations [3]. Barriers to gene flow are then required to maintain separation and prevent homogenization of diverged populations [9].

i) Initial divergence and the role of speciation genes

Although prokaryotic species evolve due to different evolutionary forces at different rates, they can also evolve in different spatial proximities, i.e. sympatry, parapatry and allopatry (Table 2, Fig. 4) [2,83]. However, in all these cases, sufficient genetic changes are required to prevent homogenization between diverged subpopulations. These genetic changes generally occur in what is considered speciation genes [83–85]. These genes can range in functionality from housekeeping to niche-associated functions and contribute substantially toward establishing genetic isolation [83–85].

Theoretically, for individuals of a population to undergo sympatric speciation (Table 2), stronger divergent selective forces are required than for allopatric speciation [7,86,87]. To date, limited studies have focussed specifically on the potential for sympatric speciation in prokaryotes, because many systematists argue that what are often interpreted as sympatric speciation events may, in fact, be occurring in different non-overlapping micro-niches

Table 2
The spatial modes of speciation.

Mode	Spatial connectedness	Predominant evolutionary forces	References
Allopatry	Speciation occurs in complete spatial or temporal separation. No gene flow occurs between populations.	Speciation can occur in the presence of strong evolutionary forces, but can also occur stochastically over a prolonged period. Due to the absence of gene flow, homogenization is prevented despite the presence of strong selective pressures.	[2,86,89,91,94]
Parapatry	Also known as mosaic sympatry. Speciation occurs in the presence of some gene flow between populations, where niches are geographically situated adjacent to each other. There is thus a region of contact between populations.	Because gene flow is not completely interrupted, selective pressures is required to prevent homogenization during gene flow. Negative epistatic interactions may act as one of the main drivers of divergence.	[2,84,89-91]
Sympatry	Speciation occurs between individuals or populations occupying the same or overlapping niches. No physical barriers to gene flow are present.	Speciation occurs in the presence of gene flow. This suggest that homogenization of populations through HGT occurs at a lower frequency than mutations leading to negative epistatic interactions of genes.	[2,9,84,89-91]

due to slight ecological adaptations within the bigger environment with intermittent gene flow between subpopulations (see below; [2,9,84,88–91]). However, some evidence for sympatric speciation in overlapping niches has been discovered in marine *Vibrio* populations, where few loci are required to ensure survival within the respective overlapping niches [9,84,90,92]. These loci thus act as speciation genes by becoming strongly differentiated between diverging populations and driving speciation despite limited differentiation at neutral loci (Fig. 4c; [9,77]). In these cases, genetic conversions of neutral loci can routinely occur between the different populations, while conversions at the adaptive loci are limited [9,77,87]. Phylogenies of neutral loci would thus still indicate reticulate evolution between populations, but phylogenies of adaptive loci will indicate divergence between the populations [9]. During the later stages of speciation, however, the rate at which genetic exchange occurs between the populations would decline, at which time differentiation between neutral loci would also occur thus emphasizing divergence between the populations [9,77].

Speciation in the presence of intermittent gene flow, due to spatial proximity (e.g. in adjacent niches with a shared contact zone), can occur between populations occupying distinct niches [2,84,89,91,93,94]. This is known as parapatric speciation (Table 2, Fig. 4b). In these circumstances, individuals occurring within the contact zone can be seen as chimeras or "hybrids" of the two parental populations [93]. These chimeric individuals would thus group with one population when analysing certain characters, while grouping with the other population when analysing different characters. Over time, these populations would diverge sufficiently so that various barrier traits (see below) would appear to maintain their separation.

In contrast to sympatric speciation, speciation occurring in allopatry (Table 2, Fig. 4a) is much more intuitive, and explanations for the process are more comprehensive [2,83,86,89,91,94]. Pioneer genes, a type of speciation gene relating specifically to ecology, are usually the main drivers for divergence during allopatric speciation [8], as these genes alter the niches employed. These genes cannot be purged from the population through selective sweeps due to their necessity for survival in the specific niche (i.e., niche-transcending genes becomes niche-specifying genes; [17,95,96]). In these cases, spatial or temporal separation act as a physical barrier to homogenizing gene flow between subpopulations. Genomic changes occurring neutrally or through selective pressures can become fixed within the population over time, allowing cohesion of the population as a whole but divergence from the parental population (which would also be evolving independently).

ii) Maintenance of separation

After initial divergence of subpopulations, gene flow between the separate subpopulations has to be limited [9]. This is achieved through various barrier traits that disrupt interspecific gene flow. In the absence of these barrier traits, homogenizing HGT can occur between the diverged subpopulations, thereby eliminating all signals for initial divergence and subsequent convergence of the subpopulations [69]. Four of the most notable barrier traits are indicated below.

a Niche alteration:

Niche-associated characteristics of individuals may be changed in a single HGT event [28,42]. Because niche-altering HGTs may occur quite frequently, divergence of subpopulations through allopatric means is not uncommon [83]. Thus, if the diverged subpopulations are not reintroduced into the same niche, the lack of proximity of individuals to one another poses an obstacle to homogenizing genetic conversions [13,97]. The continued spatial separation after initial divergence of subpopulations acts as a physical barrier to gene flow. Similarly, temporal separation of niches after initial divergence prevents convergence through homogenizing HGT [13].

b Reduced homology:

Another well-studied barrier assisting in limiting genetic conversions relates to the decrease in HGT frequency with an increase in genetic divergence [97]. In all studied cases, it has been shown that closely related individuals undergo genetic conversions at a much higher rate compared to more distantly related individuals [98,99]. This phenomenon may be due, in part, to the increased stability of the homology-dependent recombinant joint that forms during homologous recombination-based genomic integration of DNAs received via horizontal means [97,100]. Another contributing factor is the DNA mismatch repair system employed by prokaryotes to limit potentially detrimental nucleotide changes [47,97]. When a homologous, but not exact, copy of a sequence is introduced through HGT-based genetic conversions, mismatches between the newly introduced copy and the native complementary strand may cause distortions in the overall structure of the DNA. These distortions are substrates for the DNA mismatch repair systems that ultimately facilitates replacement of the original sequence with the foreign DNA [101]. The ability of DNA mismatch repair systems to restrict genetic conversions, however, appears extremely variable among taxa [97]. In *Bacillus*, a naturally competent genus, ca. 16% of the limitations to converging gene flow could be attributed to DNA mismatch repair systems [102] as opposed to the 34% observed in *S. pneumonia* [103].

c MGE host range:

The host range of HGT mediators is another factor that can limit homogenizing gene flow upon subsequent contact between diverged subpopulations [69]. Although a number of MGEs can integrate DNA into recipient genomes without recognition sites, some are extremely strict and require specific recognition sequences [10]. The host range of these MGEs can thus be altered if divergence eliminates recognition sequences from the

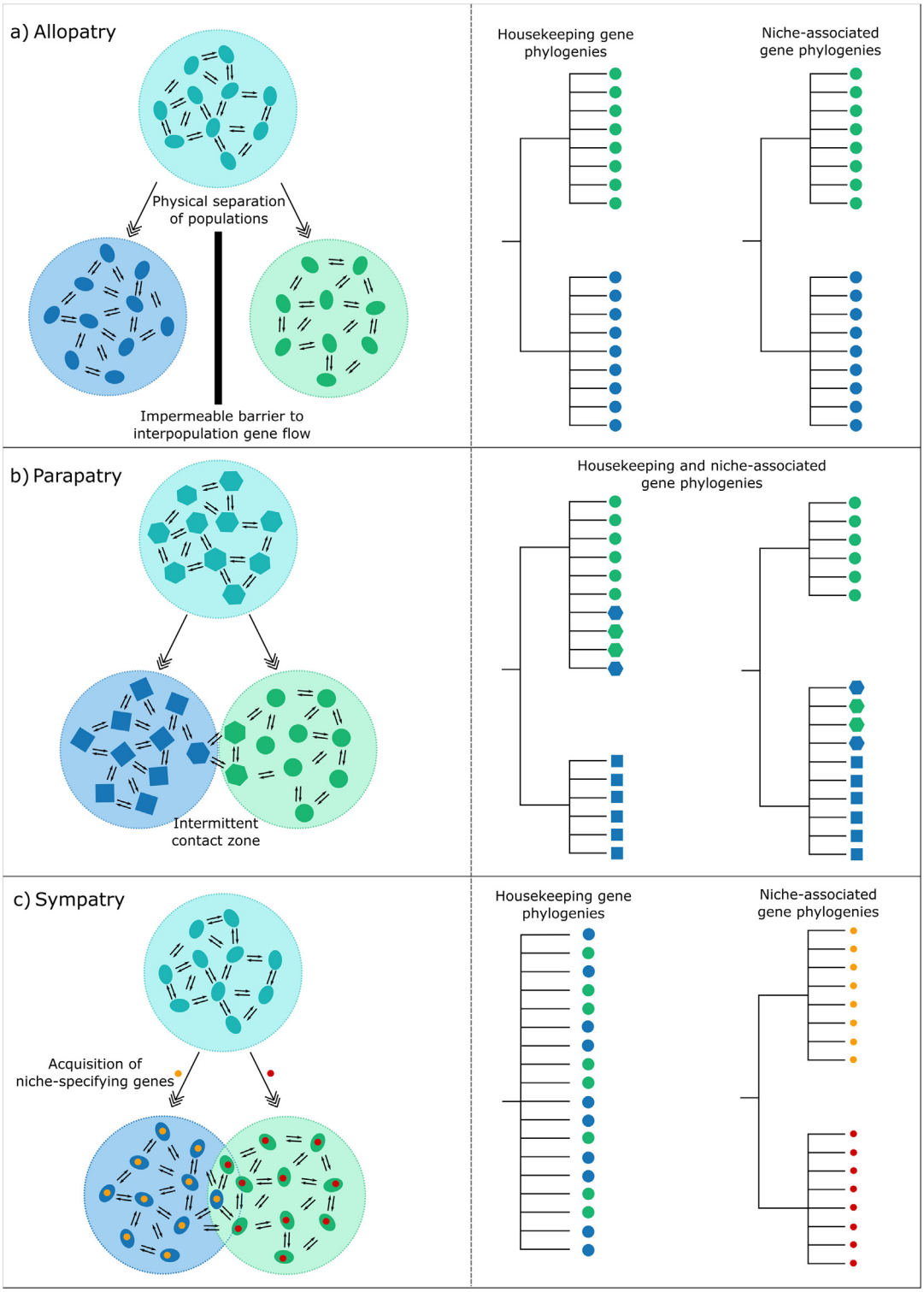

Fig. 4. Phylogenetic expectations for the different spatial modes of speciation. a) During allopatric speciation physical separation acts as an impermeable barrier to gene flow between diverged subpopulations, leading to independent evolution of housekeeping and niche-associated genes. b) Parapatric speciation is characterised by separate niches with an intermittent contact zone between diverged subpopulations. This results in some gene flow producing chimeric individuals occurring in the contact zone. In these instances, a parental population of individuals (hexagons) may diverge into two separate subpopulations (squares representing subpopulation 1 and circles representing subpopulation 2). When analysing phylogenetic trees, "hybrids" (resembling the parental population; hexagons) may group with one diverged subpopulation in some gene trees and the other subpopulation in other gene trees, independent of gene function. c) Sympatric speciation occurs when niche-specifying genes for overlapping niches develop and specialisation for the specific niches is driven by a limited number of genes. In phylogenetic trees, these genes will suggest separation of the subpopulations, which will not be evident from those encoding housekeeping functions as they are subject to continued HGT between diverged subpopulations.

genomes of previous hosts, reducing the potential shared genetic pool by eliminating the newly diverged non-host population.

d Negative epistatic interactions:

Negative epistatic interactions can be caused by either adaptive or neutral mutations in alleles that accumulate in the subpopulations during their initial divergence [2]. Notable prokaryotic

genes subject to epistasis [104–106] are informational genes, as is described in the complexity hypothesis [107,108]. Informational genes are those that are generally considered as essential for survival as they perform crucial cellular functions and often form part of gene or protein complexes [107]. Because these genes and their products usually interact with each other, they are often not horizontally transferred [107,108]. This is due to the improbability that all interacting genes occurring on non-contiguous regions in the genome will be transferred simultaneously [107,108]. Thus, if some of these genes are transferred successfully, they may not be able to interact with the necessary homologs in the new host genome and would not be able to provide the cellular processes necessary for survival [107,108]. Individuals of the diverged subpopulations undergoing genetic conversions at these loci would not be able to survive and homogenization of the subpopulations can be prevented.

Prokaryotic species concepts and the integrated model of how diversity is structured

An appropriate species concept as the basis for systematics for prokaryotes, accommodating all evolutionary forces and the variability observed in different taxa, cannot yet be agreed upon by systematists. To investigate how the integrated model presented here (Fig. 3), fits in with our current understanding of prokaryotic evolution, the most commonly referenced concepts (Table 3) attempting to consolidate the differences between the species category and a species taxon were considered [28,29,80].

Because of the tremendous effect of HGT in prokaryotic evolution, neither the adapted biological species concept of Dykhuizen and Green (1991) nor the ecotype concept of Cohan (2002) (Table 3), are sufficient for describing all prokaryotic species. The effects of both vertical inheritance and cohesive forces (intraspecific gene flow in the biological species concept and selective sweeps in the ecotype concept) [8,13,48,68,109,110] are, however, accommodated in the integrated model. Some species may be very strict in their sources for HGT, reminiscent of interbreeding conspecific individuals, and can indeed evolve mainly through vertical descent with intermittent selective sweeps occurring and acting as a cohesive force. However, highly recombinogenic species, undergoing frequent interspecific gene flow, cannot be accommodated by these concepts.

The more recently proposed "Public Goods Hypothesis" (Table 3) can fully explore and accommodate HGT [53] and readily aligns with what one would expect for a genetic continuum. It also aligns with the integrated model, which regards all species as having the potential to undergo HGT. However, the Public Goods Hypothesis is not informative regarding cohesiveness of species groups and it makes no differentiation between horizontal acquisition of DNA and vertical descent. Following this hypothesis, the genomes of all individuals (be it cells or HGT mediators) are considered as various combinations of public goods (genes) and the potential exist that any combination of genes is possible [53]. Although this limitless concept may in fact aid in the exploration of diversity space, it does not currently provide a practical means to study prokaryotic species.

Among contemporary species concepts, the one that best aligns with the prevailing views of how species are structured in diversity space (i.e., the integrated model) is De Queiroz's (2007) notion that species are "segments of separately evolving meta-population lineages" (Table 3). Under this concept, and other evolution-based concepts [73,79,80], rigid limits are not placed on how prokaryotes can or should evolve [28,80,87,111]. Also, like the integrated model, this concept accommodates all evolutionary processes and the associated heterogeneity resulting from prokaryotic speciation [80,111]. De Queiroz's concept can thus form the basis for studying prokaryotic species from an evolutionary perspective.

Practical investigation of prokaryotic diversity space requires a pluralistic approach, because as stated by Bapteste and Boucher (2008) "no single coherent explanatory system can account satisfactorily for all the diverse phenomena of life" [112]. Thus, evolution may appear ecotype-like in some taxa and 'public goods'-like in others, but probably mostly as 'segments of separately evolving meta-population lineages'. Although this perspective may alter the number of currently recognized species, this approach is the only way to obtain evolutionarily and biologically informative data about taxa, as opposed to the typical cut-off based approaches routinely used for prokaryotic species delineation [113,114]. Such a pluralistic view also recognizes the fact that species are *sui generis* in nature, because as they are kept cohesive through different evolutionary forces, at different genomic similarity levels and over different evolutionary time scales, [28,80,81,95].

Implications for prokaryote systematics

Over the past few decades, prokaryotic taxonomists have been increasingly employing evolutionary criteria for species delineation. This is evident in the need for monophyly for taxon descriptions above the species level [31,115–117]. Relationships for these ranks are inferred from conserved and shared genes and traits, excluding characters particularly prone to HGT [6,118]. Prokaryotic systematics is also moving toward genome-based phylogenetic analyses, where the signal for historic HGTs are often overshadowed by the signal for vertical descent [98,99], which limits not only their detection but also their effects on higher level taxonomy. In other words, current and historic HGT may occur between these groups, but the overarching signal for vertical inheritance in these groups will be recoverable despite reticulation [98,99].

At the species level, the effect of HGT is particularly pronounced, because it greatly influences the robustness of phylogenies and subsequent taxonomy [6,8,29,32,42]. In fact, HGT may increase the continuity in some areas of diversity space to such an extent that discrete species clusters cannot be identified [28]. Chimeric taxa with characters that overlap with those of multiple other species groups may further complicate their delineation [31]. Despite these complexities, however, some form of classification is required for practical purposes (i.e. to study, manipulate and utilize prokaryotic species) [6,8,28,32,119].

Although genome sequences provide a means to obtain a robust evolutionary-based classification system using phylogenetics, this resource also provided insight into the frequency of HGT [28,42,95]. Concerns regarding the current taxonomic system focusses largely on the lack of phenotypic cohesion, regardless of genomic cohesion, due to frequent chimerism [28,29]. To accommodate potential chimeric individuals or species, suggestions focussing on the ecological or predicted function [120,121] of organisms have been made as an alternative to the current, phylogeny-based classification system [28,29]. Following this alternative approach, taxa that are functionally (and phenotypically) more similar would be regarded as fulfilling the same functional role and would be considered as equivalents, despite not sharing common ancestry [28,29,32].

Both approaches (phylogeny-based taxonomy and functionality-based taxonomy) have merit for studying prokaryotic evolution, although only one can be implemented meaningfully for classification purposes. The current classification system, based on shared ancestry, already provides an invaluable resource in that species can be described fitting into a robust higher-level hierarchy. If one accepts that classification systems are solely

Table 3
Promising species concepts for bacteria.

Concept	Essence of the concept	Dealing with HGT	References
Biological species concept	Recombination is the cornerstone of this concept. Members of the same species would be able to exchange genetic information horizontally, while members from different species would lack this ability. Strict concordance between genealogies would be the operational criterion for this species concept.	Since the description of this concept, HGT has been recognized as a major evolutionary force in bacteria and this concept cannot accommodate HGT occurring between species.	[8,109,110,114]
Ecotype concept	Three observations for eukaryotes form the basis of this concept. i) Divergence in a population is prevented through cohesive forces. ii) Divergence is not reversible. iii) Diverged populations are distinct from one another. In bacteria, only ecotypes satisfy these requirements, thus all current species would contain multiple ecotypes that would be elevated to species level.	Although HGT is not explicitly excluded from this concept, excessive HGT between ecotypes complicates the issue. Genetic conversions may allow individuals of one ecotype to gain traits unique or essential to another ecotype, thus individuals may belong to multiple ecotypes within their lifetime.	[13,28,42,48,68]
Public goods hypothesis	All genetic material is considered to be public goods and can be incorporated in various combinations to form the genome of an organism. The requirements of public goods are that it should be non-excludable and non-rivalrous. However, some genes may be excludable from some genomes due to the required genetic background for functioning. The concept does not really consider what a bacterial species is for practical purposes and also does not provide predictive power or delineation potential.	This concept can accommodate all vehicles containing genetic information and all evolutionary forces, particularly HGT.	[53]
Meta-population lineages concept	Species are considered "segments of separately evolving meta-population lineages". This concept focuses on separate evolutionary trajectories between species. The flexibility of this concept makes it impossible to obtain operational criteria and species should be evaluated on a case-by-case basis.	All evolutionary forces can be accommodated at different evolutionary rates, and may reflect bacterial speciation most accurately.	[28,29,80,111]

manmade constructs, then one should follow the most pragmatic solution, which would be to unify the view of species with the current classification system. A first step towards such a unification process would involve recognition of the fact that prokaryotic species are essentially *sui generis*, each with their own evolutionary ages, tendencies, population dynamics and evolutionary fates.

The *sui generis* nature of prokaryotic species aligns well with the current phylogeny-based taxonomic system. In this framework, one can employ the available tools for investigating phylogenetic cohesion (see Supplementary Table S1), and use this criterion as the basis of taxonomic decisions [114,122–128]. For example, robust and reliable species clusters can be identified with genealogical concordance, which involves the use of multiple independent genealogies to identify a putative species boundary as the point where the genealogies' branching patterns become concordant [114,129–132]. Such evolution-based approaches are in stark contrast to the various similarity indices used to guide species delineation (e.g., 16S rRNA gene sequence similarity, DNA–DNA Hybridization and Average Nucleotide Identity), and which assumes the same level of cohesion among all prokaryotic species [115–117,133–141].

HGT affects all of the approaches traditionally used to investigate genetic, phenotypic and genomic cohesion (Supplementary Table S1). Some approaches are highly susceptible to producing erroneous or misleading results in the presence of HGT, as in the case of single gene datasets (e.g., 16S rRNA gene similarity and phylogenies [142,143]), physiological comparisons [144] and genomic cohesion approaches that do not consider the proportion of the genomes analysed [87,138,139]. Conversely, most of the phylogenetic cohesion approaches can overcome the spurious signal associated with HGT if sufficient data is utilised [98,126,145–148], with some approaches even providing insight into the frequency of, and genes affected by, HGT [114,122,123]. The same is also true for the widely employed multi-locus sequence analysis (MLSA) approach as it uses a limited set of genes for delineating species [126,149]. By taking into account HGT and its effects on prokaryotic evolution, taxonomic studies can be enormously enriched, because it improves our knowledge of the speciation process and how this impact the biology of the taxa being studied.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2018.10.002.

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