Conceptualizing microbe-plasmid communities as complex adaptive systems

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Highlights

- Mobile genetic elements such as plasmids and phages transfer genes between microbes, shaping the diversity and function of microbial communities. Hence, understanding the processes that govern the structure and dynamics of microbe-plasmid interactions is a fundamental goal of microbial ecology.
- Addressing this goal is challenging because communities are governed by processes that operate simultaneously at multiple levels of community organization.
- Complex adaptive systems theory, implemented via agent-based evolutionary modeling and extended by network analysis, is a promising framework to overcome these challenges.
- Using model comparison in combination with empirical data (experimental or from nature), researchers can quantify the relative importance of the key processes that shape the structure and function of microbial communities.

Key words: agent-based models | community dynamics | ecological networks | microbial ecology | mobile genetic elements

Abstract

Plasmids shape microbial communities' diversity, structure, and function. Nevertheless, we lack a mechanistic understanding of how community structure and dynamics emerge from local microbe-plasmid interactions and co-evolution. Addressing this gap is challenging because multiple processes operate simultaneously at multiple levels of organization. For example, immunity operates between a plasmid and a cell, but incompatibility mechanisms regulate coexistence between plasmids. Conceptualizing microbe-plasmid communities as complex adaptive systems is a promising approach to overcoming these challenges. I illustrate how agent-based evolutionary modeling, extended by network analysis, can be used to quantify the relative importance of local processes governing community dynamics. These theoretical developments can advance our understanding of plasmid ecology and evolution, especially when combined with empirical data.

Microbe-plasmid communities are complex and adaptive

Plasmids fuel microbe evolution, shaping microbial communities [1]. Because microbial communities affect ecosystem functions, from nutrient recycling in oceans to host physiology to disease transmission, microbe-plasmid interactions have far-reaching consequences. A hallmark example is the spread of antibiotic resistance (ABR) genes [2]. Consequently, understanding how ecological and evolutionary processes shape the **structure** (see Glossary) and dynamics of microbe-plasmid interactions is a fundamental goal of microbial ecology.

Addressing this goal is challenging because, in nature, multiple physiological, ecological, and evolutionary processes operate simultaneously at multiple **interaction levels**, generating complex networks (Figure 1, Key Figure) [3–6]. "Network thinking" [7] is necessary for two reasons. First, due to indirect effects, community functions depend on network structure rather than isolated pairwise interactions. For instance, the structure of plasmid genetic similarity networks can define the dynamics of ABR gene transmission across animal hosts [8]. Similarly, the structure of microbe-phage networks affects HGT [9]. Network structure also affects the response of microbial communities to perturbations [10], although this has not been studied in plasmids. Second, network structures contain signatures for the processes that generated them [11,12] (Figure 1). For example, the structure of sequence-similarity networks can result from mosaicism [7], and the structure of bacteria-plasmid networks is shaped by ABR-carrying plasmids [13]. In addition to being complex, microbe-plasmid communities consistently evolve at ecological time scales [6,14]. Therefore, interaction networks dynamically evolve and affect the processes that generate them [12] (Figure 2). Nevertheless, how these complex communities assemble and evolve has been understudied. Therefore, we currently lack a fundamental, mechanistic understanding of (i) how local processes govern the emergence of diversity and network structures; and (ii) the subsequent consequences of structure for functions such as plasmid transmission and community stability.

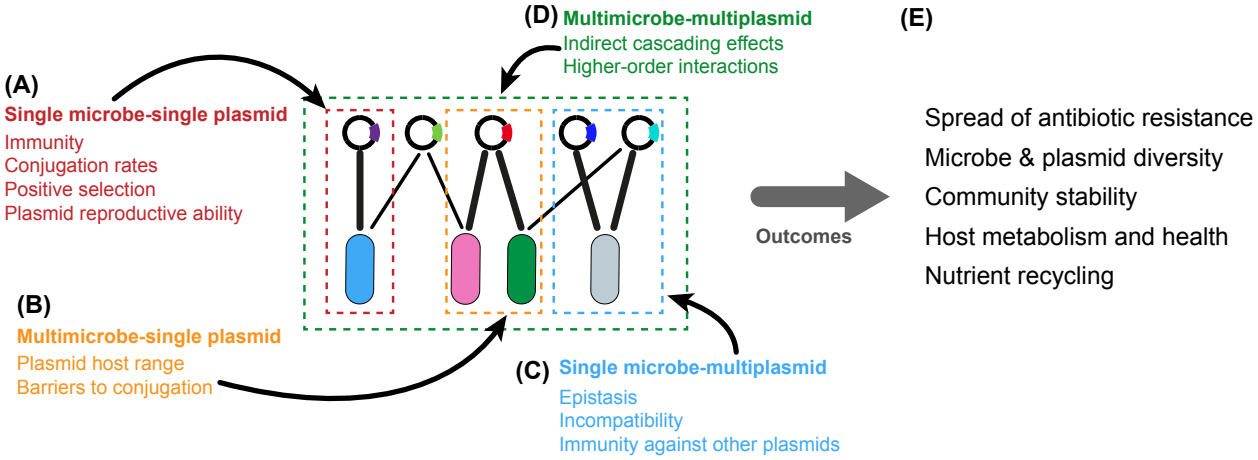

Figure 1. Levels of interaction of microbe-plasmid communities and their relevant processes. Multiple factors and processes—operating simultaneously at various levels of complexity—govern the emergence of community dynamics, diversity, and interaction networks. A representative example for each level is depicted inside the dashed boxes in panels (A)-(C). The color of each bacteria and plasmid represents a strain. The color of the dashed boxes corresponds to the interaction level processes noted. (A) Microscopic rules that operate at the single microbe-single plasmid level govern processes at higher interaction levels. For example, in (B), the prevalence of the red plasmid in the pink bacteria depends on its prevalence in the green one; for example, because the green bacteria is a reservoir host [15]. In (C), coinfection of the gray bacteria by the blue and cyan plasmids is possible if the plasmids do not exclude each other via immunity. (D) The entire interaction network must be considered to fully understand community dynamics and structure because multilevel processes result in nonlinear indirect effects and higher-order interactions. (E) Network structure and community dynamics determine ecological outcomes and functions (a few examples are stated).

Complex adaptive systems can be studied using models

These questions can be addressed experimentally. For instance, compensatory mutations maintain plasmid diversity [16], and plasmid copy number accelerates the evolution of ABR [1]. However, conducting evolutionary experiments that capture the emergence of diverse interaction networks is typically not feasible. Moreover, studying competing hypotheses requires multiple experiments, which can be costly. Models can guide experiments and are valuable because they focus on a few key processes. For instance, the spread of plasmids across microbial populations depends primarily on transmission rates, fitness cost to the host, and positive selection (e.g., antibiotics) [17]. I argue that addressing the aforementioned knowledge gaps requires evolutionary models. Such models enable studying communities as complex adaptive systems (CAS) in which **macroscopic patterns** emerge from **microscopic rules** that operate on the system's agents (Box 1). I consider plasmid entities and microbial cells as the fundamental agents on which microscopic rules, and therefore selection, operate (Figure 2). Alternative views at finer (genes as agents) or coarser (plasmid and microbe **strains** as agents [12]) scales are possible as long as the organizational scale matches the question at hand. The scale I focus on is analogous to community ecology, whereby agents are comparable to individuals in macro-organisms, and strains are analogous to species [18]. Community ecology has a rich history of studying mechanisms that underlie the emergence of diversity and structure, and drawing on this research can be beneficial to microbial ecology.

Studying microbe-plasmid communities as CAS enables the development of a predictive framework to understand the relative importance of the various processes determining their **co-evolutionary** and **eco-evolutionary** dynamics. For example, plasmid evolution is governed by multiple 'lifestyle modes' such as invasion, genome adaptation, and host range and persistence—each of which is governed by different processes and rules [14]. Although I focus on plasmids, my arguments are relevant for other transmissible mobile genetic elements (MGEs), such as temperate phages [19]. Additionally, some of the ideas I raise are inspired by work on lytic phages, and I mention those where appropriate. I first synthesize knowledge on the microscopic rules that govern the structure and dynamics of microbe-plasmid communities at increasing interaction levels (Figure 1, Figure 2A). I then suggest methodology to study the emergence of structure.

Box 1. Complex adaptive systems

In complex adaptive systems (CAS), macroscopic patterns emerge from the collective behavior of agents that interact and adapt according to a set of microscopic rules [20]. Macroscopic patterns can be described at the system level, such as the structure of species interaction networks, nutrient fluxes, and community stability (Figure 1A). Microscopic rules refer to local interactions between the system's components (e.g., conjugation, immunity, competition) and to factors that affect these components (e.g., mutation, trait distributions) (Figure 2A).

The word 'adaptive' indicates that evolutionary processes change and select components based on emerging patterns; that is, components adapt in response to emerging dynamics and interactions [21]. For instance, plasmids evolve mechanisms that allow them to increase host range when new microbial strains appear. Evolution requires trait heterogeneity; therefore, there should be diverse components. In a seminal paper, Levin [21] argued that CAS require sustained diversity of systems components (e.g., many plasmid/microbe strains). Diversity can be an initial condition; for example, one can initialize a model with a high diversity of agents. However, diversity can also be an emerging property [12,22,23]. For example, in host-parasite systems, frequency-dependent selection that provides an advantage to rare variants is a driving force generating and maintaining parasite and host diversity and emerges from competition between parasite variants for hosts [23]. CAS are characterized by dynamical feedback between macroscopic patterns and microscopic rules (Figure 2). Such feedback leads to non-linearity; that is, local interactions can change as the system evolves. For example, a plasmid's ability to infect a microbe can be reduced if that microbe evolves a defense mechanism due to an interaction with another plasmid.

Evolutionary ABMs are a tool to model CAS. Studies in microbial ecology, which aim to identify fundamental principles that dominate community dynamics and structure [24] or raise hypotheses to explain the structure of communities (e.g., phage-bacteria interaction networks [11]) implicitly consider microbial communities as CAS. However, there is a paucity of studies using evolutionary ABMs in a CAS framework to test specific hypotheses. An example of this approach is shown in Figure I.

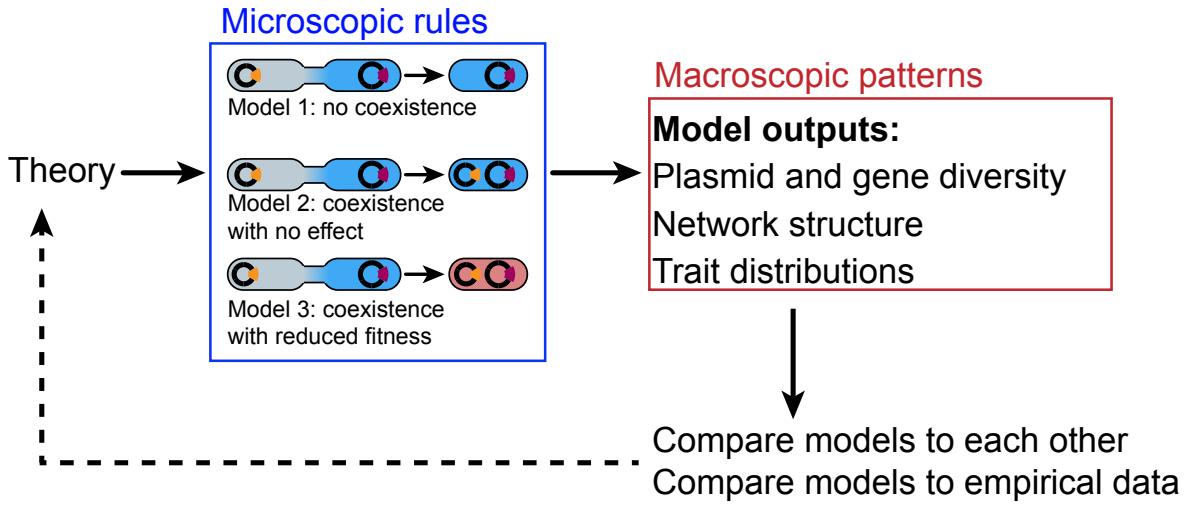

Figure I. Figure I. Using evolutionary ABMs. Given a theory, multiple models can be explicitly coded and compared. In the three models presented, plasmids move between cells via conjugation. Plasmids cannot coexist (model 1), can coexist with no effect on host fitness (model 2), or can coexist while reducing host fitness (model 3). Model outputs can be compared to quantify effects and test hypotheses. In addition, comparison to empirical data can aid in identifying key microscopic rules that operate in nature. Knowledge gained from these comparisons can improve existing theory (dashed line).

Microbe-plasmid interactions are shaped at multiple levels of interaction complexity

Plasmids are extra-chromosomal double-stranded DNA molecules. While they can be transferred to microbial cells via vertical transmission, **horizontal gene transfer (HGT)** is a significant force driving their persistence. Plasmids contain backbone genes that encode essential functions allowing them to control their replication and transmission (e.g., via conjugation), though some cannot self-mobilize) [25,26]). These processes exploit host machinery, reducing host growth rates and incurring fitness costs. Therefore, microbes contain non-adaptive (e.g., restriction-modification) and adaptive (e.g., CRISPR) immune mechanisms [27]. Immunity (microbes) and the ability to evade immunity (plasmids) are traits under constant selection pressure, which mediate the ability of plasmids to infect microbial cells. Therefore, immunity is a key molecular mechanism mediating co-evolutionary dynamics and interactions.

However, some plasmids contain accessory genes that encode additional traits that affect their host cell. Accessory genes, such as those providing increased nutrient uptake rates in nutrient-poor environments, can confer an advantage to the host [5]. If the plasmid carries beneficial accessory genes, the benefits to the host can outweigh the costs [5]. The fitness cost imposed by plasmids, coupled with potential plasmid loss during cell division (Figure 2A) and the fact that beneficial accessory genes can integrate into the host chromosome, should hamper plasmid persistence. This reasoning contradicts their frequently observed long-term persistence, sometimes even in the absence of positive selection [28]—a puzzle termed the "plasmid paradox". Multiple ecological and evolutionary solutions to this paradox have been described, including high transmission rates, compensatory mutations that ameliorate plasmid cost, and variation in plasmid cost across microbial strains [29].

The microscopic rules operating at the single microbe-single plasmid level manifest in processes at higher interaction levels. These processes, in turn, feed back to affect the local rules. In nature, plasmids are embedded in diverse microbial communities (Figure 1B). Variation in host competence and susceptibility can affect plasmid persistence. For example, Hall et al. [15] have shown that the plasmid pQBR57 was not maintained in monocultures of *Pseudomonas putida* populations (a non-favorable host). However, when co-cultured with *P. fluorescens* (a favorable host), the plasmid was present in *P. putida* because *P. fluorescens* served as a reservoir species. Supported by a mathematical model, these experimental results indicate that inter-strain transmission can promote plasmid persistence in an unfavorable host species. In a later study, Kottara et al. [30] found that pQBR57 was more prevalent in the focal host *P. fluorescens* SBW25 in a monoculture than when cultured in a mixture containing other *Pseudomonas* strains. In the presence of non-focal host strains, the infectivity of pQBR57, a broad host range plasmid, is diluted in the focal host. Barriers to conjugation or other mechanisms of HGT can arise due to incompatibility between a plasmid and a microbe (e.g., immunity) or factors such as spatial distribution. Therefore, at the multimicrobe-single plasmid level, plasmid host range and barriers to HGT are two key factors determining microbe-plasmid interactions.

Multiple plasmid types can exist in the same cell (Figure 1C). From the plasmid perspective, coinfection drives competition for host resources [31]. This intense selective pressure could lead to the evolution of exclusion mechanisms. For instance, plasmids can prevent the entry, or hamper the reproduction, of competing plasmids via multiple mechanisms such as surface exclusion or anti-plasmid CRISPR immunity [32,33]. Moreover, plasmids from the same incompatibility group (Inc) have similar replication machinery, preventing them from stably coexisting within the same host cell [26]. On the other hand, coinfection can lead to fitness benefits for all resident plasmids. Coinfection can also result in gene exchange via recombination, driving plasmid evolution [1]. From the host perspective, within-cell plasmid interactions can alter fitness costs (epistasis) [34]. Hence, the cost of harboring multiple plasmids can differ from the additive cost of harboring each alone [35,36]. While the molecular processes of plasmid coinfection have been studied [37], its ecological underpinnings and outcomes are only now being explored [4,38,39]. For example, coinfecting plasmids can co-transfer or reduce each other's conjugation rates via "fertility inhibition" [31,39].

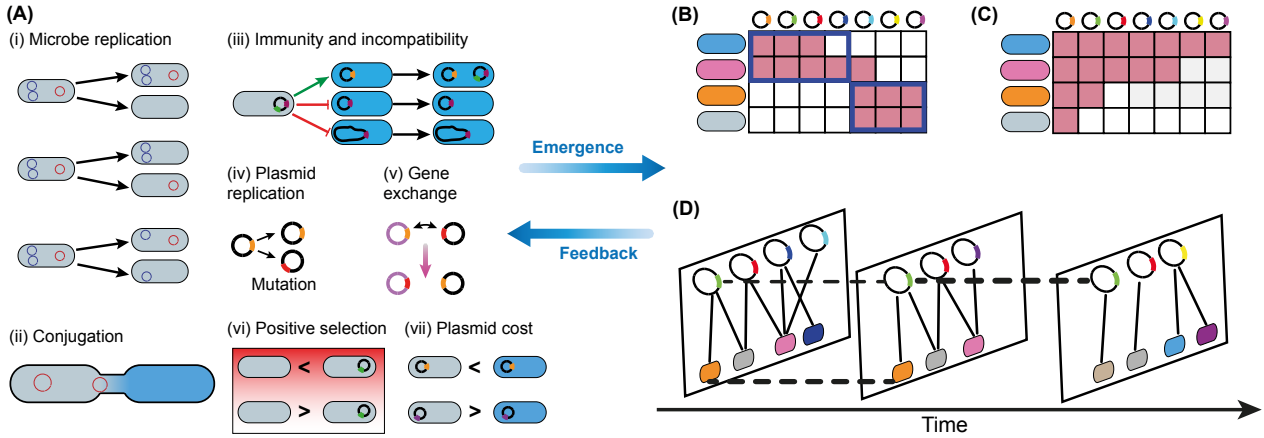

Figure 2. Microbe-plasmid communities as complex adaptive systems. (A) Main processes and interactions (microscopic rules). *(i)* Upon microbe replication, plasmids are vertically passed to the daughter cells with possible plasmid loss. *(ii)* Conjugation is a common mechanism of plasmid-controlled movement between cells. *(iii)* Plasmid transmission or long-term persistence fails (red line) if the recipient cell has a plasmid of the same incompatibility group or if the recipient cell or its resident plasmids are immune. Identical dark purple segments depict matching incompatibility or immunity. *(iv)* Plasmids replicate and can mutate upon replication. *(v)* Plasmids can recombine to exchange genes. *(vi)* The fitness of a cell bearing a plasmid with a beneficial gene (depicted in green) can be greater than that of microbes not bearing the plasmid when the environment is unfavorable (depicted by red in the gradient). *(vii)* Plasmids can have different costs in different microbes. The orange plasmid is costly in the blue bacteria, and the purple plasmid in the gray. (B) and (C) are matrix representations of, respectively, modular (blue rectangles depict two modules) and nested networks that encode infections of microbes by plasmids. (D) Model output as a temporal multilayer network (each layer is a time step). Interlayer edges (dashed) connect agents to themselves in the next layer, and their width is proportional to the change in agent frequency (only three are shown for clarity). Notice how the identities of agents and interactions change over time. This evolution of topology cannot be captured using monolayer networks.

The multimicrobe-multiplasmid level is the most relevant, yet challenging, to study

Given that all the processes above operate in tandem, multimicrobe-multiplasmid systems (Figure 1D) are the most relevant and informative for understanding the dynamics of microbe-plasmid communities. Nevertheless, they are also the most challenging to study due to their high diversity and complexity and because they encompass all the processes at lower interaction levels. While this level of interaction encompasses the most significant knowledge gap, its investigation will allow a thorough understanding of how microbe-plasmid community dynamics operate in nature. Ample evidence for this claim comes from community ecology. In a multihost-multiparasite community, indirect effects cascade across host-parasite interaction networks to affect parasite interactions [40] and the evolution of both hosts and parasites [41]. Moreover, in ecological communities, **higher-order interactions** are abundant and strongly affect community dynamics [42]. For instance, a microbe strain can serve as a source population to a plasmid that also infects another microbe strain [15].

The high diversity that characterizes microbes and plasmids implies variation in trait distributions [43,44]. In microbes, variation in susceptibility to infection and the ability to support plasmid replication (competence) underlies their differential abilities to support plasmid population and plasmid transmission [15,30]. For example, plasmid costs vary between strains, indicating variation in host competence [45]. Plasmids exhibit variation in traits such as the range of hosts they can infect [15,30] and their ability to mobilize themselves [25]. For instance, some plasmids possess conjugation machinery genes, while those lacking such genes can "hitchhike" on the former [25,35]. Current studies rarely accommodate the vast microbe and plasmid strain and trait diversity observed in nature. Considering diversity is important, because coinfection and multimicrobe transmission result in **nonlinear effects** [30,34]. Under conditions of high plasmid diversity, there is an immense poten-

tial for plasmid-type combinations in microbe cells and, subsequently, variation in the outcomes of plasmid-plasmid interactions. For instance, plasmid transfer rates can be reduced or enhanced in the presence of a coinfecting plasmid in the microbe population [38]. Similarly, high microbial diversity can support plasmid persistence on non-preferred hosts [15].

Box 2. A network approach to studying microbe-plasmid interactions

Networks are an ideal framework to describe and analyze the complexity of systems with many interacting entities and are often used in microbial systems [7,8,46]. Network structure influences stability and function [10], and provides a signature for the processes that generate the network.

A host-parasite network is an ecological network describing 'who infects whom'. These are bipartite networks where interactions are allowed between nodes from two distinct sets (hosts and parasites) but not within each set. In community ecology, host-parasite networks have been extensively studied [40]. In microbial ecology, microbe-phage infection networks—a private case of host-parasite networks—have been studied, focusing on two structural properties: modularity and nestedness [11,47,48]. In a modular network (Figure 2B), microbes and phages are partitioned into groups of densely-interacting strains—a structure describing patterns of specialization and niche construction—and modules represent bacterial niches on which phages can grow [12]. In a nested network (Figure 2C), specialist microbes are infected by subsets of phages that, in turn, infect the more generalist microbes, and specialist phages infect subsets of the microbes infected by the more generalist phages [11]—a structure describing patterns of specialization. The emergence of these structures is affected by spatial and phylogenetic scales [49] and microbe-phage coevolutionary dynamics (arms-race) [48].

In CAS, the network structure at each time step depends on the one(s) before forming a temporal network. Network evolution can be described using multilayer networks [50,51]. Each layer contains nodes and intralayer links representing the structure at a particular time. Interlayer links connect nodes at different layers and encode processes that operate between layers according to the research question [52]. For instance, if the number of microbial cells is a primary factor in determining microbe-plasmid interactions, then interlayer edges can encode changes in the frequency of strains (Figure 2D).

Although it is possible to analyze temporal dynamics without interlayer edges [12], their inclusion can reveal hidden patterns. One example comes from the malaria parasite *Plasmodium falciparum*. A vast pool of gene variants in local parasite populations can generate concomitant diversity of parasite genomes. Competition for hosts structures these combinations in a way that limits their genetic overlap. This 'limiting similarity' is expected to result in a modular structure in which strains from the same module occupy the same niche in host immune space. While modules were not apparent in static networks [23], they were observed in temporal multilayer networks in which interlayer links describe the competitive effect of a parasite strain at one time on other strains at the next time point [51].

Modeling evolving microbe-plasmid communities

A central question in plasmid ecology regards spreading dynamics in microbial populations because plasmids carry ABR genes. Therefore, the dominant approach to modeling microbe-plasmid interactions has been population-level models (PLMs) [17,53]. PLMs use differential equations to model the average state of a population in time. They can incorporate interactions between plasmids and microbes and between plasmids (e.g., coinfection [39]), and are primarily designed to quantify the effects of different parameters (e.g., loss and transmission rates) on plasmid spread. However, PLMs cannot be used to model CAS because they describe population averages rather than agents. In addition, strain diversity is provided at the onset and does not evolve.

Incorporating evolution is possible using evolutionary agent-based models (ABMs) in which mi-

crobes and plasmids are agents. In this framework, diversity and interaction structure emerge from rules encoded in the model [12,23,53,54] (Figure 2). Moreover, ABMs allow the explicit representation of individual stochastic variation while being computationally tractable [55]. This is particularly important for plasmids because some only exist in low copy numbers [56]. Evolutionary ABMs are **process-based** models [57], which explicitly implement theory to test competing hypotheses (Box 1). For example, one can test the effect of different kinds of plasmid-plasmid incompatibility mechanisms on microbe-plasmid interactions by comparing the output of models that include different rules. Moreover, process-based models allow for generating projections, which can then be compared to empirical data. This, in turn, enables the development of predictive models. Nevertheless, developing ABMs that include eco-evolutionary and co-evolutionary dynamics is challenging due to the multiple interaction levels and processes involved (as described above). Incorporating these processes may require many parameters. As with any modeling approach, researchers should focus on the parameters (and interaction levels) most relevant to their hypotheses. ABMs also require advanced computing skills and take longer to run. These may be the primary reasons why ABMs have rarely been used to describe microbe-plasmid dynamics [53,58,59].

Analyzing emerging structures using networks

ABMs generate, as in nature, highly diverse communities with complex interaction networks that change in time [12] (Box 2). Some community functions can be analyzed directly from model outputs. For instance, it is possible to quantify the number of microbial strains that evolved ABR. However, it is particularly insightful to study the emerging networks for two reasons. First, comparison of model outputs with empirical data can aid in identifying processes that shape natural communities. For instance, two prevalent structural properties in bacteria-phage networks are **modularity** and **nestedness** [47,48] (Figure 2B,C). These patterns have dynamical implications for phage transmission [12,48,49], but the processes that lead to their emergence are still a matter of debate (Box 2). In plasmids, a recent experiment at a multimicrobe-single plasmid level showed that the number of plasmid interactions and their strength (plasmid prevalence) depends on the effect of the plasmid on microbe growth rate [60]. Another study at the multimicrobe-multiplasmid level showed that ABR-carrying plasmids infected more bacterial species, increasing the connectivity of the plasmid-bacteria network [13]. Second, even when studying functions directly, the mechanism behind them requires an understanding of the network (Figure 1E). For instance, phage outbreaks that are observed in model outputs result from particular evolving network structures, which were also identified in empirical data [12].

The temporal dynamics inherent to CAS generate evolving networks. A solution to this challenge is multilayer networks [50] (Figure 2D, Box 2). In a temporal multilayer network, each layer represents the structure of the community at a given time point (layer). A dedicated analysis incorporating the structure within and between the layers can reveal temporal patterns of evolutionary changes hidden in static networks [51]. For example, the evolution of microbe susceptibility to plasmids can be captured by quantifying temporal changes in the number of plasmids to which a microbe is connected. A particular insightful network property is modularity. In multilayer networks, unlike in monolayer networks, modules can cross layers and therefore contain microbes and plasmids from multiple time points [51]. Modules then delineate groups of microbes and plasmids that interact strongly with each other in time. Hence, module birth and death dynamics can indicate an intrinsic evolutionary time scale.

Concluding remarks

Conceptualizing microbe-MGE interactions as CAS will advance our understanding of the mechanisms that govern their structure. This theoretical understanding is a fundamental step in predicting and controlling the function of microbial communities—for example, engineering communities with a particular microbial composition that minimizes the spread of foreign genes. Previous works have focused on modeling the transmission dynamics of plasmids to understand the spread of ABR genes in low diversity systems [17]. However, strain transmission is ultimately a result of ecological and

evolutionary rules that operate at the agent level in highly diverse communities. A combination of process-based ABMs and network analysis is a promising approach to close this gap by investigating emergent evolutionary and transmission dynamics (see Outstanding Questions). Process-based models are particularly beneficial because they allow identifying the key factors that govern the emergence of variation in specific traits. For instance, does variation in the susceptibility of microbes to plasmids result from fast plasmid adaptation or under-developed immunity? In addition, models are general and, therefore, applicable to MGEs other than plasmids.

Theoretical results should be tested with evolutionary experiments. While it is extremely challenging to reach the high diversity and complexity described by models in experimental systems, it may not always be necessary [15,30,60,61]. Evolutinoary ABMs can guide future research by allowing researchers to focus on mechanisms relevant to the interaction levels they investigate. Finally, a comparison of model outputs to natural systems is also necessary. Advanced metagenome techniques that allow comprehensive sampling of the mobilome and microbiome [13,44] should prove useful for that purpose. Shifting the theoretical approach from modeling non-evolving communities to CAS should lead, in my opinion, to a conceptual leap in studies of microbe-MGE communities.

Outstanding questions

- Can we identify key processes that underlie the composition and structure of communities observed in nature or in controlled experiments? For instance, when does HGT contribute to diversity more than variation in plasmid copy number?
- Under which conditions do plasmid stability mechanisms (those that solve the plasmid paradox) evolve? Are those affected by microbe-plasmid interaction structure?
- Can we predict the emergence of network structures such as modularity and nestedness via local processes?
- Is there a fundamental difference in the rules that govern the community structure of MGEs with different life histories (e.g., plasmids vs temperate or lytic phages)?
- What is the relative importance of neutral processes (e.g., variation in abundance and pure population dynamics) vs selection in the assembly of microbe-plasmid communities?
- Can we identify the relative importance of factors / rules that determine evolving community functions? For example, microbes evolving the ability to acquire plasmids vs plasmids' ability to evolve multiple copies in degerming spread of ABR.

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Declaration of Interests

There are no interests to declare.

Glossary

- **Co-evolutionary dynamics:** Temporal changes in the genetic composition and associated phenotypes of interacting agents (e.g., plasmids and microbes).
- **Eco-evolutionary dynamics:** Temporal changes in macroscopic patterns (e.g., diversity) that result from the interplay between ecological (e.g., population dynamics) and evolutionary (e.g., mutation) processes.
- **Higher-order interactions:** Effect of an agent on the interaction between two (or more) other agents. For instance, when the infection of a cell by a plasmid is facilitated or impeded by a plasmid already residing in the cell.
- **Horizontal gene transfer (HGT)**: The movement of genes between organisms not via cell division. Main HGT mechanisms are transduction, transformation and conjugation. HGT typically involves entities such as phages or plasmids that carry the genes between organisms.
- **Interaction levels:** The hierarchical organization of the way community members interact: from an interaction between a single microbial cell and a single plasmid entity to interactions between multiple microbes and plasmids.
- **Interaction structure:** Also termed network structure. The pattern by which network links are distributed across nodes. In the context of networks, 'interaction' is a network link: a plasmid infecting a microbe.
- **Macroscopic patterns:** The collective properties, dynamics, and behavior that emerge from local interactions and processes. For example, the diversity of the microbiome emerges from processes such as mutation, HGT and microbe-plasmid interactions.
- **Microscopic rules:** Interactions and processes that operate between the system's agents. For instance, rate of mutation, the number of copies a plasmid can produce, and the ability of a plasmid to penetrate a microbial cell.
- **Modularity:** A network structure in which the network is partitioned into groups (modules) of nodes (e.g., strains) that interact strongly with each other and weakly with nodes from other groups.
- **Nestedness:** In ecological networks, nestedness is a structure in which specialists interact with proper subsets of the species interacting with the more generalists.
- **Nonlinear effects:** Broadly speaking, a nonlinear effect occurs when the outcome of microbe-plasmid interactions is not proportional to the values of their traits, and therefore typically cannot be directly predicted. When community dynamics are nonlinear, interactions change as the system evolves.
- **Process-based models:** Models that explicitly represent knowledge or hypotheses regarding the factors and processes that determine the relationships between agents and their evolution. This is in contrast to phenomenological models (e.g., Lotka Volterra-type models), in which the mechanism underlying the relationship between variables is not explicitly stated. Process-based models are encoded using simulations of dynamical processes. Note that like with any other kind of model, not all factors that operate can, or should be, represented.
- **Strain:** A genetically-unique microbe or MGE. It is generally assumed that strains differ in some phenotype relevant for the system.

References

1. Rodríguez-Beltrán, J. et al. (2021) Beyond horizontal gene transfer: the role of plasmids in bacterial evolution. Nat. Rev. Microbiol. 6, 347–359

2. Gillings, M.R. (2017) Lateral gene transfer, bacterial genome evolution, and the Anthropocene. Ann. N. Y. Acad. Sci. 1389, 20–36

3. Hernández-Beltrán, J.C.R. et al. (2021) Mathematical models of plasmid population dynamics. Front. Microbiol. 12, 606396

4. Lopez, J.G. et al. (2021) Modeling the ecology of parasitic plasmids. ISME J. 15, 2843–2852

5. San Millan, A. and MacLean, R.C. (2017) Fitness costs of plasmids: a limit to plasmid transmission. Microbiol Spectr 5

6. Garoña, A. and Dagan, T. (2020) Darwinian individuality of extrachromosomal genetic elements calls for population genetics tinkering. Environ. Microbiol. Rep. 13, 22–26

7. Corel, E. et al. (2016) Network-thinking: graphs to analyze microbial complexity and evolution. Trends Microbiol. 24, 224–237

8. Shapiro, J.T. et al. (2023) Multilayer networks of plasmid genetic similarity reveal potential pathways of gene transmission. ISME J DOI: 10.1038/s41396-023-01373-5

9. Popa, O. et al. (2017) Phylogenomic networks reveal limited phylogenetic range of lateral gene transfer by transduction. ISME J. 11, 543–554

10. Coyte, K.Z. et al. (2015) The ecology of the microbiome: Networks, competition, and stability. Science 350, 663–666

11. Weitz, J.S. et al. (2013) Phage–bacteria infection networks. Trends Microbiol. 21, 82–91

12. Pilosof, S. et al. (2020) The network structure and eco-evolutionary dynamics of CRISPR-induced immune diversification. Nat Ecol Evol 4, 1650–1660

13. Risely, A. et al. (2022) Antimicrobial resistance genes predict plasmid generalism and network structure in wastewater. bioRxiv DOI: 10.1101/2022.05.11.491481

14. Hülter, N. et al. (2017) An evolutionary perspective on plasmid lifestyle modes. Curr. Opin. Microbiol. 38, 74–80

15. Jordt, H. et al. (2020) Coevolution of host-plasmid pairs facilitates the emergence of novel multidrug resistance. Nat Ecol Evol 4, 863–869

16. Leclerc, Q.J. et al. (2019) Mathematical modelling to study the horizontal transfer of antimicrobial resistance genes in bacteria: current state of the field and recommendations. J. R. Soc. Interface 16, 20190260

17. Pascual, M. (2020) High non-neutral Diversity and High-Dimensional trait space in Pathogen Populations and ecological Communities. In Unsolved Problems in Ecology (Dobson, A. et al., eds), Princeton University Press

18. Harrison, E. and Brockhurst, M.A. (2017) Ecological and evolutionary benefits of temperate phage: what does or doesn't kill you makes you stronger. Bioessays 39, 1700112

19. Smillie, C. et al. (2010) Mobility of plasmids. Microbiol. Mol. Biol. Rev. 74, 434–452

20. Orlek, A. et al. (2017) Plasmid classification in an era of whole-genome sequencing: application in studies of antibiotic resistance epidemiology. Front. Microbiol. 8, 182

21. Labrie, S.J. et al. (2010) Bacteriophage resistance mechanisms. Nat. Rev. Microbiol. 8, 317–327

22. Wein, T. et al. (2019) Emergence of plasmid stability under non-selective conditions maintains antibiotic resistance. Nat Commun 10, 2595

23. Brockhurst, M.A. and Harrison, E. (2022) Ecological and evolutionary solutions to the plasmid paradox. Trends Microbiol. 30, 534–543

24. Hall, J.P.J. et al. (2016) Source–sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 113, 8260–8265

25. Kottara, A. et al. (2021) The dilution effect limits plasmid horizontal transmission in multi-species bacterial communities. Microbiology 167

26. Getino, M. et al. (2017) PifC and Osa, Plasmid weapons against rival conjugative coupling proteins. Front. Microbiol. 8, 2260

27. Garcillán-Barcia, M.P. and de la Cruz, F. (2008) Why is entry exclusion an essential feature of conjugative plasmids? Plasmid 60, 1–18

28. Pinilla-Redondo, R. et al. (2020) Type IV CRISPR-Cas systems are highly diverse and involved in competition between plasmids. Nucleic Acids Res. 48, 2000–2012

29. Dionisio, F. et al. (2019) Interactions between plasmids and other mobile genetic elements affect their transmission and persistence. Plasmid 102, 29–36

30. San Millan, A. et al. (2014) Positive epistasis between co-infecting plasmids promotes plasmid survival in bacterial populations. ISME J. 8, 601–612

31. Gama, J.A. et al. (2020) Plasmid interactions can improve plasmid persistence in bacterial populations. Front. Microbiol. 11, 2033

32. Bouet, J.-Y. et al. (2007) Plasmid partition and incompatibility–the focus shifts. Mol. Microbiol. 65, 1405–1414

33. Gama, J.A. et al. (2017) Conjugation efficiency depends on intra and intercellular interactions between distinct plasmids: Plasmids promote the immigration of other plasmids but repress co-colonizing plasmids. Plasmid 93, 6–16

34. Igler, C. et al. (2022) Plasmid co-infection: linking biological mechanisms to ecological and evolutionary dynamics. Philos. Trans. R. Soc. Lond. B Biol. Sci. 377, 20200478

35. Runghen, R. et al. (2021) Network analysis: ten years shining light on host-parasite interactions. Trends Parasitol. 37, 445–455

36. Pilosof, S. et al. (2014) Host-parasite network structure is associated with community-level immunogenetic diversity. Nat. Commun. 5, 5172

37. Sanchez-Gorostiaga, A. et al. (2019) High-order interactions distort the functional landscape of microbial consortia. PLoS Biol. 17, e3000550

38. Kottara, A. et al. (2018) Variable plasmid fitness effects and mobile genetic element dynamics across Pseudomonas species. FEMS Microbiol. Ecol. 94

39. Kav, A.B. et al. (2020) Unravelling plasmidome distribution and interaction with its hosting microbiome. Environ. Microbiol. 22, 32–44

40. Alonso-Del Valle, A. et al. (2021) Variability of plasmid fitness effects contributes to plasmid persistence in bacterial communities. Nat. Commun. 12, 2653

41. Zwanzig, M. (2021) The ecology of plasmid-coded antibiotic resistance: a basic framework for experimental research and modeling. Comput. Struct. Biotechnol. J. 19, 586–599

42. Gross, T. and Sayama, H. (2009) Adaptive Networks. In Adaptive Networks: Theory, Models and Applications (Gross, T. and Sayama, H., eds), pp. 1–8, Springer Berlin Heidelberg

43. He, Q. et al. (2018) Networks of genetic similarity reveal non-neutral processes shape strain structure in *Plasmodium falciparum*. Nat. Commun. 9, 1817

44. Nardini, J.T. et al. (2021) Learning differential equation models from stochastic agent-based model simulations. J. R. Soc. Interface 18, 20200987

45. Paulsson, J. (2003) Plasmids as stochastic model systems. in Fluctuations and Noise in Biological, Biophysical, and Biomedical Systems, 5110, pp. 125–131

46. Cuddington, K. et al. (2013) Process-based models are required to manage ecological systems in a changing world. Ecosphere 4, art20

47. Harrison, E. et al. (2015) Bacteriophages limit the existence conditions for conjugative plasmids. mBio 6, e00586

48. Hall, J.P.J. et al. (2017) The evolution of plasmid stability: Are infectious transmission and compensatory evolution competing evolutionary trajectories? Plasmid 91, 90–95

49. Flores, C.O. et al. (2011) Statistical structure of host-phage interactions. Proc. Natl. Acad. Sci. U. S. A. 108, E288–97

50. Fortuna, M.A. et al. (2019) Coevolutionary dynamics shape the structure of bacteria-phage infection networks. Evolution 73, 1001–1011

51. Beckett, S.J. and Williams, H.T.P. (2013) Coevolutionary diversification creates nested-modular structure in phage-bacteria interaction networks. Interface Focus 3, 20130033

52. Newbury, A. et al. (2022) Fitness effects of plasmids shape the structure of bacteria-plasmid interaction networks. Proc. Natl. Acad. Sci. U. S. A. 119, e2118361119

53. Pilosof, S. et al. (2017) The multilayer nature of ecological networks. Nat Ecol Evol 1, 0101

54. Pilosof, S. et al. (2019) Competition for hosts modulates vast antigenic diversity to generate persistent strain structure in *Plasmodium falciparum*. PLoS Biol. 17, e3000336

55. Blazanin, M. and Turner, P.E. (2021) Community context matters for bacteria-phage ecology and evolution. ISME J. 15, 3119–3128

56. Carmichael, T. and Hadžikadić, M. (2019) The Fundamentals of Complex Adaptive Systems. In Complex Adaptive Systems: Views from the Physical, Natural, and Social Sciences (Carmichael, T. et al., eds), pp. 1–16, Springer International Publishing

57. Levin, S.A. (1998) Ecosystems and the biosphere as complex adaptive systems. Ecosystems 1, 431–436

58. Westra, E.R. et al. (2017) Mechanisms and consequences of diversity-generating immune strategies. Nat. Rev. Immunol. 17, 719–728

59. Konopka, A. et al. (2015) Dynamics in microbial communities: unraveling mechanisms to identify principles. ISME J. 9, 1488–1495

60. Faust, K. et al. (2012) Microbial co-occurrence relationships in the human microbiome. PLoS Comput. Biol. 8, e1002606

61. Hutchinson, M.C. et al. (2019) Seeing the forest for the trees: Putting multilayer networks to work for community ecology. Funct. Ecol. 33, 206–217