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Quantitative analysis of horizontal gene transfer in complex systems

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Horizontal gene transfer (HGT) plays a significant role in rapidly propagating diverse traits throughout bacterial populations, thereby accelerating natural evolution and leading to complex community structures. Critical gene transfer rates underlying these occurrences dictate the efficiency and speed of gene spread; these rates are often highly specific to HGT mechanism and environmental context, and have historically been challenging to reliably quantify. In this review, we examine recent works that leverage rigorous quantitative methods to precisely measure these rates in a variety of settings beginning with *in vitro* studies and advancing to *in situ* measurements; we emphasize contexts where quantification across multiple scales of complexity has led to fundamental biological insights. Finally, we highlight the applications of these measurements and suggest potential methodological advances to improve our understanding.

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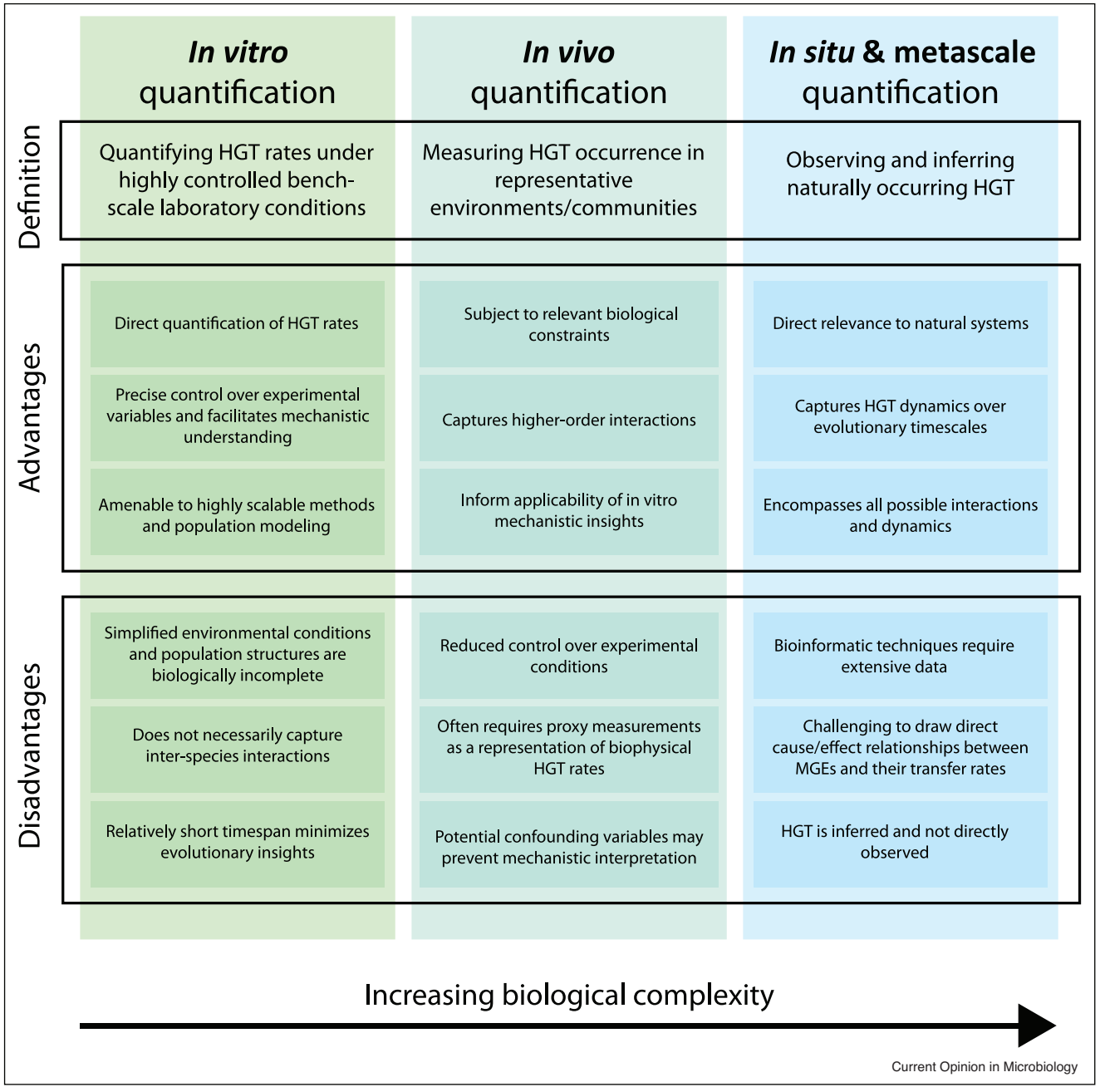
Introduction

The discovery of **horizontal gene transfer (HGT)** in the early 20th century was a milestone for classic microbiology. By the 1950s, the three main mechanisms of HGT – conjugation, transformation, and transduction – had been identified [1–3] (Figure 2), though other less common mechanisms have since been described. In each case, **mobile genetic elements (MGEs)** are taken up by recipient cells that enjoy the benefits and suffer the costs of associated gene maintenance and expression. These MGEs take diverse forms including plasmids, integrative conjugative elements, insertion sequences, transposable elements, and gene cassettes/integrons [4]. Moreover, MGEs encode diverse cargo including metabolic traits [5,6], virulence factors [7,8], and antibiotic resistance genes [9,10]. In turn, the prevalence of particular MGEs, and the population-level advantages derived thereof, are highly dependent on their associated transfer rates. These rates are often modulated by specific cellular systems and factors, including gene content, donor and recipient strain/species, cell physiology, and environmental conditions [11,12•]. Here, we highlight recent studies that utilize emergent quantitative techniques to measure these transfer rates in increasingly complex biological contexts: *in vitro*, *in vivo*, and *in situ*. We emphasize computational and systems biology approaches and briefly discuss the limitations associated with each scale of measurement. Finally, we discuss tangible applications of such insights and potential strategies to fill current knowledge gaps, with an emphasis on microbial risk assessment, an exciting example with public health applications. Overall, we demonstrate that although each context has unique drawbacks and strengths, all are invaluable in understanding HGT; ultimately, their combination generates fundamental, actionable insights.

Quantifying HGT rates under different levels of complexity

MGE transfer rates are universal to all scales and mechanisms of HGT; nonetheless, each is subject to different biophysical constraints. For example, conjugal plasmid transfer rates (conjugation efficiencies) are highly dependent on donor cell physiology, whereas recipient cell competency is crucial to transformation efficiency. Given the range of potentially interdependent HGT rate-modulating factors, a detailed understanding requires complementary insights across multiple levels of complexity. Below, we highlight key works that quantify gene transfer rates across these contexts; whether *in vitro*, *in vivo*, or *in situ*, each scale of investigation seeks to balance tradeoffs between parallel objectives of quantitative precision and biological relevance (Figure 1).

Figure 1

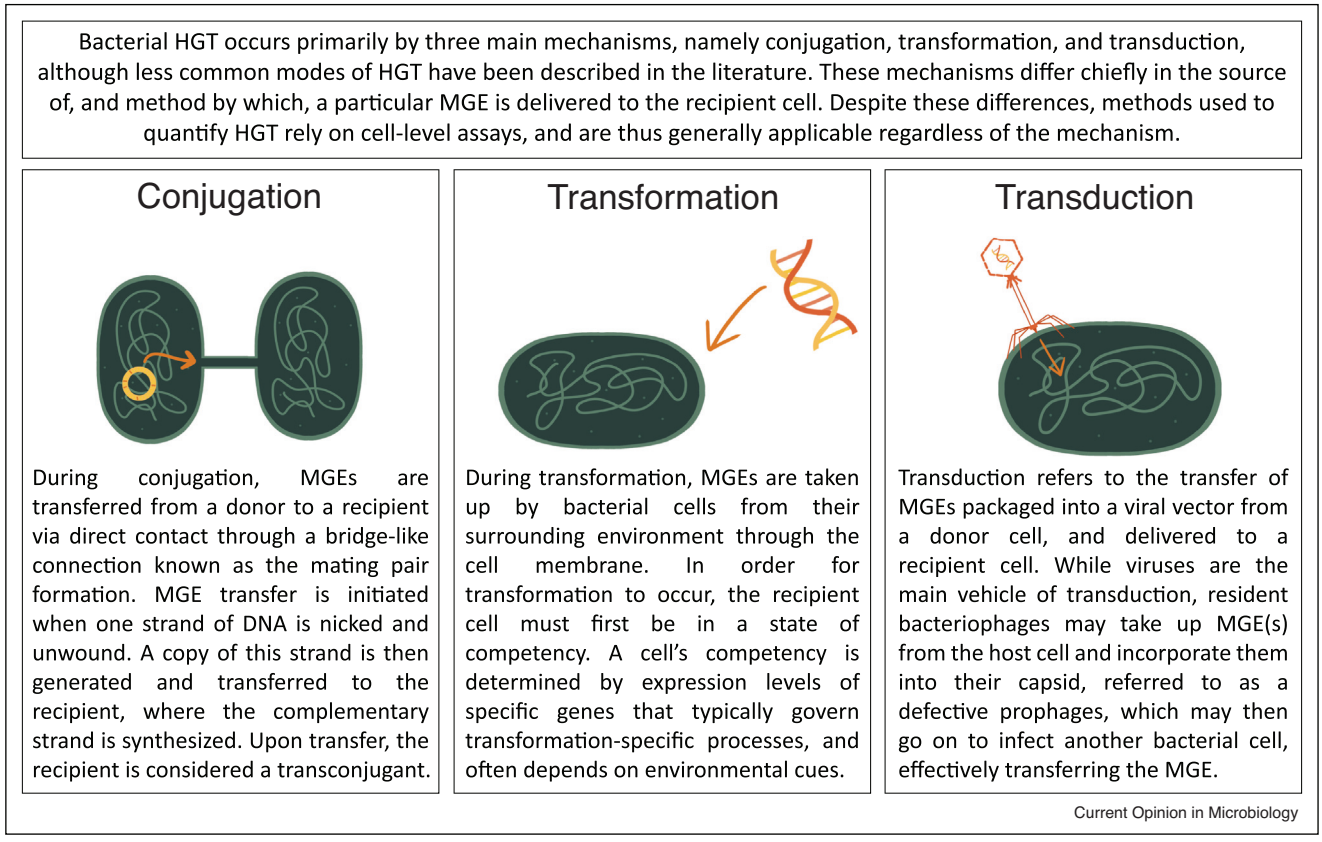


Quantification scales of HGT.
The three experimental scales at which to measure HGT are shown from left to right, with increasing biological complexity: *in vitro*, *in vivo*, and *in situ* and metascale quantification. The definition of each is shown at the top, and the top advantages and disadvantages of each experimental design are shown below.

In vitro quantification of HGT

By *in vitro*, we specifically refer to laboratory experiments under highly controlled conditions. These approaches rely on quantitative experimental techniques including microfluidics [13], qPCR [14], and flow cytometry [15,16], amongst others, to isolate and quantify the biophysical transfer process [17•]. Despite being inherently simplified reflections of natural HGT, they are ideal environments to measure specific rates and isolate modulating factors. For example, Perez-Mendosa and de la Cruz used a novel luminescence assay, wherein visible light emitted as a result of plasmid transfer was

Figure 2



Mechanisms of horizontal gene transfer.

directly proportional to the conjugation efficiency, in order to quantify the transfer rate of nearly 24 000 *Escherichia coli* mutants [18], each harboring a distinct genetic modification; results indicated specific recipient strain characteristics that impacted conjugation efficiency. In these and other cases [19], rapid phenotyping facilitated a broad exploration of relevant genetic space. *In vitro* quantitative rigor is not unique to high throughput techniques. For example, although several studies demonstrated that antibiotics may increase conjugation efficiency (e.g. rate of plasmid transfer), Lopatkin *et al.* used selective agar plating to show that when the growth of donors and recipients were examined, antibiotics did not increase conjugation efficiencies for ten representative antibiotics and nine plasmids [13]. Importantly, they estimated the transfer rate as a bimolecular kinetic process, minimizing the impact of selection dynamics and other confounding variables. The experimenters further determined that conjugation dynamics are impacted by diverse antibiotic-mediated selection, wherein different drug treatments can favor bacteria containing a resistance plasmid, thereby altering overall population structure post-conjugation.

Because of their controlled nature, *in vitro* experiments are also amenable to mathematical modeling, which can grant insight into both gene transfer rates and resulting dynamics [20]. For example, Cooper *et al.* [21] observed that *Acinetobacter baylyi* predation increased HGT via transformation due to the release of DNA; they used a mathematical model to determine conditions favoring HGT, which suggested that predation may serve as a microbial adaptation strategy. Computational modeling studies can also lead to counterintuitive insights not immediately evident from *in vitro* work: Van Dijk *et al.* [22••] used an *in silico* model of *in vitro* conjugation to show that although HGT is metabolically costly, sufficiently rapid transfer can maintain minimally beneficial genes within a population. Complementing this work, Prensky *et al.* devised a novel method to quantify the transient metabolic burden imposed by a conjugative plasmid [23]; computational modeling of this phenomenon revealed that accounting for the time window immediately following conjugation was critical to accurately describe long-term population dynamics. These results are highly relevant to clinical settings, where León-Sampedro utilized mechanistic modeling to describe the

interplay between inter-patient and intra-patient antibiotic resistance dissemination [24]. Collectively, in addition to isolating specific transfer processes, these studies illustrate that biophysical insights must be combined with environmental and growth contexts to provide a complete, informative biological picture.

In vivo quantification of HGT

In contrast to their *in vitro* counterparts, *in vivo* approaches trade a degree of controllability to incorporate the complexity inherent in natural systems. Often, *in vivo* works utilize engineered/well-characterized strains in native environmental matrices (including animal models [25,26•] and biofilms [27,28], amongst others) and/or naturally representative bacterial communities [27,29] thereby potentially confirming *in vitro* results. For example, Ohlsen *et al.* [30] quantified gene transfer using filter mating on sewage agar plates and in liquid sewage to show that antibiotics do not increase plasmid conjugation rates in raw sewage. These and other [31] results confirm Lopatkin's *in vitro* findings and more strongly inform our understanding of HGT-mediated antibiotic selection dynamics in increasingly natural environments.

In many *in vivo* systems, direct transfer rate quantification is prohibitively complex; in these cases, proxy measures can be used to derive underlying rate parameters. For example, a series of compelling studies utilized engineered derivatives of the RP4 plasmid [27,29,32,33] to seed traceable plasmids into native bacterial communities; although kinetic transfer rates could not be reliably measured, modeling population dynamics elucidated the relative permissiveness (transconjugants generated per potential recipient) of diverse recipient strains. This is a common theme in *in vivo* studies: the use of multiple strains and species in mixed populations is a powerful approach to observe dynamics in an environment more closely resembling nature [34].

The use of increasingly complex strains, populations, and environments also enables *in vivo* studies to interrogate naturally occurring mechanisms and environmental factors that modulate, and in some cases, inhibit, the transfer of genes. For example, Domenech *et al.* demonstrated that chemical competence-blockers significantly inhibited the transformation efficiency of pathogenic strains, without affecting host growth or antibiotic efficacy, within a mouse infection model [35]; these results were consistent with *in vitro* experiments, and could eventually be used to mitigate resistance spread. Moreover, Nordgard *et al.* [36] showed that the transformation efficiencies of *A. baylyi* were reduced 2–6 orders of magnitude in the mouse gastrointestinal tract. In another setting, Lécuyer *et al.* [28] demonstrated that transfer rates of the integrative conjugative element ICEBs1 between *Bacillus subtilis* strains were significantly elevated in biofilm communities. These results further highlight that *in vivo* HGT transfer rates must be interpreted in conjunction with complementary *in vitro* studies to build a definitive understanding of specific host–microbe–environmental relationships.

In situ and metascale quantification of HGT

In the pursuit of quantitative insights, *in vitro* and *in vivo* studies necessarily admit varying degrees of abstraction, chief among them their relatively brief timespan of interest; population-level impacts of HGT manifest far beyond the immediate implications of gene transfer. To that end, *in situ* and metascale studies of HGT emphasize how MGE dissemination can affect bacterial population structure and phylogeny on evolutionary timescales. For example, multiple studies have directly connected both bacterial transduction and conjugation to increased community diversity; these works demonstrate that elevated gene transfer rates increase the likelihood of pathogenicity [37] and serve as a potential offensive mechanism against the human immune system [38]. Critically, these studies exemplify complex outcomes that can potentially be predicted with relatively simple rate parameters. These successes notwithstanding, the direct quantification of specific biophysical transfer rates remains challenging in *in situ* settings; to that end, bioinformatic approaches use phylogenetic reconstructions to estimate evolutionary rates (e.g. over longer periods of time) as a function of recombination events [39] and dispersal of MGEs [40]; these indirect methods are particularly critical in environments wherein unknown/unpredictable commingled populations interact [41]. Current research focused on wastewater treatment plants (WWTP) represents a prime example of *in situ* analysis in HGT contexts. Indeed, debate remains regarding the extent of HGT, including host ranges, rates, and the environmental factors that control them. While Munk *et al.* [42] pointed to evidence from two Danish WWTPs that the prevalence of HGT in WWTPs is likely comparable to soil, examination of three WWTPs in Hong Kong by Yin *et al.* [43] suggested that the potential for HGT was much higher than in soil. In attempting to overcome the limitations of specific experimental settings, metagenomic sequencing is a powerful tool for the exploration of the gene content of microbial communities, without *a priori* selection of target genes or hosts, and is a promising approach towards untangling such questions. For example, Dai *et al.* [44] applied nanopore sequencing to demonstrate that the relative abundance of plasmid-associated ARGs was significantly lower in activated sludge environments compared to the upstream influent sewage. Additional studies (e.g. through MetaCompare [45] and NanoARG) have synthesized ARG annotations across various MGEs to develop empirical 'resistome risk' scores; these metrics, in concert with bioinformatic approaches for example, the least-common-ancestor neural network approach applied in PlasFlow [46], can predict taxonomic host range and

therefore help identify hot spots for further study, including direct *in vitro* and *in vivo* HGT rate measurements.

Applications

Distilling the complexity of HGT, regardless of the mechanism, environment, and component species, into a defined set of transfer rates, is particularly advantageous from a systems biology perspective. Although this goal has yet to be realized, several aforementioned studies have incorporated insights from multiple scales of complexity to build phenomenological models applicable to plant, animal, and human health, as well as environmental intervention and risk assessment [47••,48]. This latter field, often termed microbial risk assessment (QMRA), combines hazard identification, exposure assessment, dose response analysis, and risk characterization to relate pathogen occurrence in various exposure scenarios (e.g. drinking water, food, or clinical environments) to a probability of infection, illness, and/or death. To date, QMRA for antimicrobial resistance has been limited by difficulties in predicting HGT impacts in both the environment and human body [49]. To that end, Chandrasekaran *et al.* [50] recently proposed a novel dose response model incorporating HGT effects; their results suggest that HGT plays a potentially significant role when cell densities were $\sim 10^{10}$ cells/mL, although these effects were dependent on the conjugation efficiency, assumed to be 10^{-11} mL/cells-day. Similarly, Njage and Buys [51] modified exposure estimates derived for a population consuming lettuce contaminated with extended spectrum beta-lactamase-resistant *E. coli*, given conjugative AmpC gene transfer frequencies developed from *in vitro* experimental data. Using a sensitivity analysis, the authors identified the HGT conjugation frequency as one of the influential variables in a probabilistic Monte Carlo risk model. In combination, these assessments indicate a need for (1) computational frameworks for integrating HGT rates into risk models as well as (2) information for populating HGT rate parameters as a function of relevant sets of conditions. These and similar studies are exciting emerging frontiers in microbiology.

Perspectives

Despite the many layers of complexity that inevitably hamper rigorous HGT transfer rate measurements, these parameters ultimately represent kinetic processes, as in any enzymatic reaction. In reality, a myriad biological components and processes modulate these rates (and therefore their reliability and predictive power). Although *in vitro*, *in vivo*, and *in situ* approaches each present unique (dis)advantages to disentangle these factors (Figure 1), we are only recently beginning to appreciate the untapped potential of applying all three in tandem. Increasingly, the integration of diverse quantitative metrics with sufficient informational context provides opportunities for powerful frameworks including machine learning/artificial intelligence (ML/AI); these approaches are optimized to infer relationships between seemingly disparate data and crystallize a predictive relationship that often is impossible to ascertain from limited data sets (e.g. individual studies). To generate relevant (and interpretable) insights, the synthesis of multiple scales and types of data (ranging from biochemical assays to phylogenetic relationships) is critical; *in vitro*, *in vivo*, and *in situ* studies together provide complementary information necessary to generate successful and applicable results [52]. Furthermore, these frameworks potentially enable longitudinal predictions of population dynamics *in situ*, allowing researchers to directly design and test for example, interventional strategies. Indeed, given the rapid scaling and technical advances in genomic sequencing, we envision that future HGT studies will close the complexity loop. The studies will pair bioinformatic-derived evolutionary insights with *in vitro* strain characterization to estimate fundamental biophysical rates. By combining results across data scales, these efforts will yield relevant mechanistic insights that could then be confidently translated to future clinical and environmental applications.

Conflict of interest statement

Nothing declared.

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