Estimating the rate of plasmid transfer in liquid mating cultures

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Abstract

Plasmids are important vectors for the spread of genes among diverse populations of bacteria. However, there is no standard method to determine the rate at which they spread horizontally via conjugation. Here, we compare commonly used methods on simulated data, and show that the conjugation rate estimates often depend strongly on the time of measurement, the initial population densities, or the initial ratio of donor to recipient populations. We derive a new 'end-point' measure to estimate conjugation rates, which extends the Simonsen method to include the effects of differences in population growth and conjugation rates from donors and transconjugants. We further derive analytical expressions for the parameter range in which these approximations remain valid. All tools to estimate conjugation rates are available in an R package and Shiny app. The result is a set of guidelines for easy, accurate, and comparable measurement of conjugation rates and tools to verify these rates.

1 Introduction

Plasmids are extra-chromosomal, self-replicating genetic elements that can spread between bacteria via conjugation. They spread genes within and between bacterial species and are a primary source of genetic innovation in the prokaryotic realm [1, 2]. Genes disseminated by plasmids include virulence factors, heavy metal and antibiotic resistance, metabolic genes, as well as genes involved in cooperation and spite [2, 3, 4, 5]. To understand how these traits shape the ecology and evolution of bacteria [6], it is of fundamental importance to understand how plasmids spread.

The abundance of a plasmid in a population is determined by two factors: (i) the horizontal transmission of plasmids between neighbouring bacteria (i.e. conjugation) and (ii) the vertical transmission of a plasmid with its host upon cell division (i.e. clonal expansion). Plasmid conjugation requires physical contact between donor cells (D), carrying the plasmid, and recipient cells (R), to create transconjugant cells (T), i.e. recipients carrying the plasmid [1]. The transconjugants then further contribute to the transfer of the plasmid to recipients. The conjugation rates from transconjugants can be substantially higher due to transitory derepression of the conjugative pili synthesis [7], and because transconjugant and recipient cells are the same species with the same restriction modification systems [8, 9]. In addition, the rates of clonal expansion of D, R, T populations can differ strongly, especially when the plasmid is transferred across species boundaries [8].

Given the importance of plasmid spread, it is surprising that there is no generally accepted method to quantify the amount of conjugation that occurs between bacterial populations. Differences between conjugation assays are dictated by the variety of biological systems in which conjugation occurs (e.g. different species require different growth medium, some plasmids require solid matrices for conjugation). All conjugation assays have in common that the donor and recipient cells are cultured together in or on a specific growth medium for a certain amount of time t. After this time, the population densities of the populations D, R, and T are measured. However, assays differ in the experimental system used - e.g. well-mixed liquid cultures, filters, plates, the gut of vertebrate hosts [8, 10, 11]; the duration of the assay t - from 1 hour to multiple days [12, 13]; and the way population densities are measured - e.g. through selective (replica) plating, or flow cytometry [10, 14, 15]. Differences in the output of such conjugation assays are then further exacerbated when the measured population densities are related to the amount of conjugation that occurred. Indeed, there is no consensus on what to call this quantity: commonly used phrases include conjugation frequency [16, 17], plasmid transfer rate constant [18, 19], or transfer efficiency [14, 15]. More than 10 different methods to quantify conjugation are currently in use (see Table 1).

Many methods are based on the ratio between population densities, such as T/D or T/R, to quantify the fraction of transconjugants at the end of the conjugation assay [12]. However, these measures vary as a function of the initial population densities, the initial donor to recipient ratio, and the length of the conjugation assay [13, 18]. Thus, experimental results reported with such measures are not comparable between studies without detailed information on the experimental conditions [10, 13]. In addition, this ratio is not only determined by a plasmid's conjugation rate, but also by its clonal expansion [14]. As such, the resulting measurements are not *a priori* comparable across experimental conditions that could affect the growth rate, including differing nutrient conditions [20], recipient species [8, 12, 21], temperatures [16], and (sublethal) antibiotic exposure [17]. This limits the predictive power of conjugation proficiency when expressed as a ratio of population densities [14].

Population dynamic models were developed specifically to disentangle the influence of horizontal and vertical plasmid transmission on final population density. In 1979, Levin et al. showed that conjugation in well-mixed liquid cultures can be accurately described with the mass action kinetics also used to describe chemical reactions [19]. They described a method to estimate the conjugation rate from bacterial population densities using linear regression in the exponential or stationary growth phase [19]. This method was developed further by Simonsen et al. [18], who derived an 'end-point' formula for the conjugation rate. This method requires a single measurement of D, R and T population densities at the end of the conjugation assay, as opposed to time-course data.

Although the Simonsen method is widely regarded as the most robust method available to estimate plasmid conjugation rates [13], thirty years after its publication in 1990 an astounding variety of methods is still in common use (see Table 1). One can speculate whether this slow adoption of the Simonsen method has been because of a sense of unease with the model-based formulation, the minor amount of extra work involved in measuring the population growth rate, or the power of habit in using population density based methods. In addition, all current methods, including the Simonsen method, have the drawback that they do not account for differences in growth rates between strains, nor in differences in conjugation stemming from donors or transconjugants. Fischer et al. [22] extended the Simonsen model along these lines, but their approach requires time course measurements and a fitting procedure which is sensitive to the initial values of the optimisation. Thus, there is a clear need to reiterate the drawbacks of population density based methods, and to lower the barrier to widespread use of better population dynamics based alternatives.

Here, we show the limitations of some existing measures of conjugation proficiency on simulated data, including their dependence on measurement time point, as well as the initial population den-

sities and ratios. To mitigate these limitations, we extend the Simonsen model to include the effects of differential population growth and conjugation rates from donors and transconjugants. For this extended model we derive a new end-point formula as well as the critical time within which these approximations are valid. We show how our extended model compares to the original Simonsen model as a function of differences in the growth and conjugation rates. To facilitate the calculation of conjugation rates from experimental data and to allow testing whether these were measured within the critical time, we provide population dynamic models and conjugation rate estimation methods in a publicly available R Shiny app. The result is a set of guidelines for easy, accurate, and comparable measurement of conjugation rates and tools to verify these rates.

Measure	**Units**	**Mating Culture**	**Name**
$\frac{T}{R_0}$	dimensionless	Plate [12]	Exconjugant frequency [12]
$\frac{T}{R}$	dimensionless	Filter [23]	Gene transfer frequency [23]
$\frac{T}{D}$	dimensionless	Liquid [16, 24]; Filter [17, 21, 23]	(Plasmid) transfer frequency [21]; Gene transfer frequency [23]; Conjugation frequency [16, 17], Recombinant yield [24], Plasmid transfer efficiency [14]
$\frac{T}{N}$	dimensionless	Liquid [20]	Conjugation frequency based on total bacterial count [20]
$\frac{T+D}{R}$	dimensionless	Liquid microcosms [25]	Plasmid prevalence [25]
$\frac{T+D}{N}$	dimensionless	Soil microcosms [26]	Frequency of plasmid carriage [26]
$\frac{T}{R+T}$	dimensionless	Liquid [8, 25], Mouse gut [8, 11]	Proportion of transconjugants [11, 25], Fraction of transconjugants (in recipient population) [8]
$\log_{10}\left(\frac{T}{\sqrt{DR}}\right)$	dimensionless	Liquid [27]	(Logarithm of) Conjugation rate [27]
$\frac{T}{DR}$	$\frac{\text{ml}}{\text{CFU}}$	Filter [28]	Transconjugant frequency [28]
$\frac{\psi_{max}}{N(b)-N(a)}\ln\left(\frac{D/N+T/R\vert_b}{D/N+T/R\vert_a}\right)$	$\frac{\text{ml}}{\text{CFU·hour}}$	Liquid (batch and chemostat) [19]	Transfer rate constant [19]
$\frac{T}{DR\Delta t}$	$\frac{\text{ml}}{\text{CFU·hour}}$	Liquid [4]	Conjugation efficiency [4]
$\psi_{max}\ln(1+\frac{TN}{RD})\frac{1}{(N-N_0)}$	$\frac{\text{ml}}{\text{CFU·hour}}$	Liquid [18], Plate [10, 13]	(Plasmid) Transfer rate [10, 18], Plasmid transfer efficiency [15], Conjugation rate per mating pair [11], Conjugation coefficient [22]

Table 1: Measures of conjugation proficiency and plasmid prevalence or spread reported in the literature. Here D, R, T stands for the population density of donors, recipients, and transconjugants at the time point of measurement, N is the total population density $N = D + R + T$, N_0 is the initial total population density, R_0 is the initial population density of recipients, ψ_{max} is the maximum growth rate of the mating culture. The measures $(T + D)/R$ or $(T + D)/N$ are primarily used to test plasmid stability in the population, rather than plasmid invasion from rare or conjugation per se.

2 Materials and Methods

Models

The Simonsen Model (SM)

Simonsen et al. [18] developed a model (the SM) that estimates the conjugation rate from a single end-point measurement of population densities of the conjugating populations (D, R, T), as well as the joint growth rate (ψ_{max}) of these populations. This model includes resource competition between the populations, and the elegant mathematical solution critically requires the assumption that both growth and conjugation have the same functional dependency on the resource concentration. The SM implicitly assumes that conjugation does not occur during the stationary phase. The dynamical equations are given by:

$$\dot{D} = \psi(C)D \tag{2.1}$$

$$\dot{R} = \psi(C)R - \gamma(C)(T + D)R \tag{2.2}$$

$$\dot{T} = \psi(C)T + \gamma(C)(T + D)R \tag{2.3}$$

$$\dot{C} = -\psi(C)(D + R + T)e \tag{2.4}$$

where the designations D, R, T stand for donors, recipients, and transconjugants respectively, $\psi(C) = \psi_{max}\frac{C}{C+Q}$ is the growth rate, $\gamma(C) = \gamma_{max}\frac{C}{C+Q}$ is the conjugation rate, C is the resource, and e is the conversion factor of resource into cells.

From this model, Simonsen et al. [18] derived that at any time point during the experiment the following relation holds:

$$\gamma_{max} = \psi_{max} \ln(1 + \frac{TN}{RD})\frac{1}{(N - N_0)} \tag{2.5}$$

where $N = D + R + T$ is the total population density at the measurement time point, N_0 is the initial population density, and the growth rate ψ_{max} should be determined from the conjugating population during the phase of exponential population growth.

The Extended Simonsen Model (ESM)

The SM makes two implicit simplifying assumptions. First, it assumes that donors, recipients and transconjugants all have the same growth rate. Second it assumes that the conjugation rate from donors to recipients (γ_{Dmax}) and from transconjugants to recipients (γ_{Tmax}) is the same. Both of these assumptions will not generally be justified. The extended Simonsen model (ESM) thus extends the SM to reflect population specific growth rates ($\psi_{Dmax}, \psi_{Rmax}, \psi_{Tmax}$) and conjugation rates ($\gamma_{Dmax}, \gamma_{Tmax}$). The dynamical equations are:

$$\dot{D} = \psi_D(C)D \tag{2.6}$$

$$\dot{R} = \psi_R(C)R - (\gamma_T(C)T + \gamma_D(C)D)\,R \tag{2.7}$$

$$\dot{T} = \psi_T(C)T + (\gamma_T(C)T + \gamma_D(C)D)\,R \tag{2.8}$$

$$\dot{C} = -\,(\psi_D(C)D + \psi_R(C)R + \psi_T(C)T)\,e \tag{2.9}$$

where $\psi_X(C) = \psi_{Xmax}\frac{C}{C+Q}$ are the population specific growth rates (subscript X stands for D, R, T), and $\gamma_Z = \gamma_{Zmax}\frac{C}{C+Q}$ are the conjugation rates from donors or transconjugants (subscript Z stands for D, T).

The Approximate Extended Simonsen Model (ASM)

We can simplify the equations for the ESM (eqs. 2.6-2.9) by assuming that the growth and conjugation rates are constant until the resource C is gone and switch to zero in stationary phase. This assumption allows one to drop the equation for the resource C as long as the stationary phase has not yet been reached. The dynamical equations of the Approximate Extended Simonsen Model (ASM) then become:

$$\dot{D} = \psi_{Dmax}D \tag{2.10}$$

$$\dot{R} = \psi_{Rmax}R - (\gamma_{Tmax}T + \gamma_{Dmax}D)\,R \tag{2.11}$$

$$\dot{T} = \psi_{Tmax}T + (\gamma_{Tmax}T + \gamma_{Dmax}D)\,R \tag{2.12}$$

Assuming that initially the dynamics of the recipient population are dominated by growth, i.e. $\psi_{Rmax}R >> \gamma_{Tmax}TR + \gamma_{Dmax}DR$, and that the transconjugant population is not yet dominated by conjugation

from transconjugants, i.e. $\psi_{Tmax}T + \gamma_{Dmax}DR >> \gamma_{Tmax}TR$, we obtain that the conjugation rate γ_{Dmax} at a time point t is given by (see supplementary materials for detailed derivation):

$$\gamma_{Dmax} = (\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax})\frac{T(t)}{D(t)R(t) - D_0R_0e^{\psi_{Tmax}t}} \tag{2.13}$$

This estimate of the conjugation rate based on the ASM can be used instead of the Simonsen endpoint formula (eq. 2.5) when the growth rates and conjugation rates differ between populations. It is valid as long as the approximate solutions are good approximations to the full ODE. In the supplementary materials we derive the critical time beyond which this approximation of the full ODE is not sufficient anymore, and the ASM end-point formula starts to break down.

3 Results

Population based methods depend sensitively on the experimental conditions

To study the merits of different measures used to quantify conjugation, we test the behaviour of the most common ones on simulated bacterial population dynamics. To this end, we simulate the population dynamics using the extended Simonsen model with resource dynamics (ESM) to include a maximum of biologically relevant detail (see Fig. 1A). Figure 1B shows that population density based measures vary over many orders of magnitude, depending on when the population densities are measured. Given the simulated cost of plasmid carriage, the T/D estimate is higher than $T/(R+T)$, although both would give (approximately) the same result if the growth rate of the D and R populations were the same. The measure $\log(T/\sqrt{DR})$ is relatively stable as a function of the measurement time. However, it is negative as long as T is smaller than D and R, and one has to take the absolute value to allow comparison with the other conjugation measures. The measure T/DR performs almost as well as the populations dynamics based measures (SM / ASM). One can also see that the dimensionless population density based measures are many orders of magnitude larger than conjugation rates estimated using population dynamic models, as the latter are typically reported in ml $\cdot$ CFU^{-1}h^{-1}.

As an example of the population-density based measures, we investigate the behaviour of the T/D method on simulated data. Figure 2 shows that T/D varies multiple orders of magnitude as a function of the initial population densities and donor to recipient ratios. This variation is independent of the

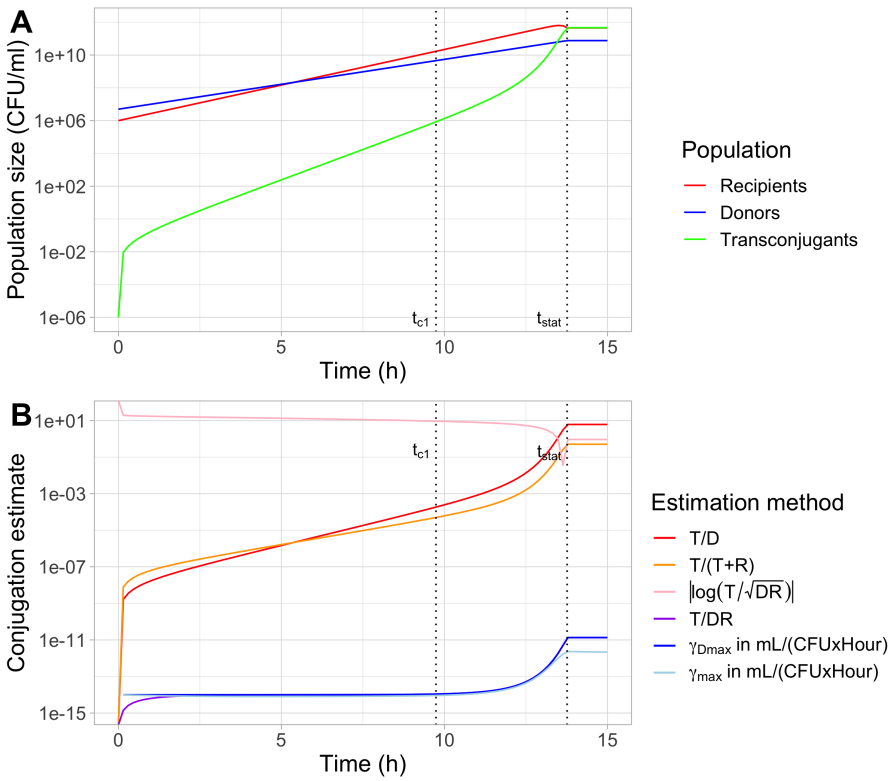

Figure 1: Impact of the time point of measurement on the magnitude of conjugation rate estimates. Panel **(A)** shows the simulated population dynamics; panel **(B)** shows the corresponding conjugation estimates according to several population density and population dynamics based methods. The SM estimate is denoted by γ_{max} and the ASM estimate by γ_{Dmax}. The T/DR and γ_{Dmax} methods are partially overlaid. The vertical dotted lines indicate the first critical time, t_{c1}, at which the contribution of conjugation events from transconjugants becomes substantial, and the time t_{stat} when stationary phase is reached (see supplementary materials). The parameters illustrate a cost of plasmid carriage, and a higher rate of conjugation from transconjugants to recipients than from donors: initial population densities $R_0 = 1 \cdot 10^6$ CFU/mL, $D_0 = 5 \cdot 10^6$ CFU/mL; initial resource concentration $C_0 = 10^{12}$ μg/mL; growth rates $\psi_{Tmax} = \psi_{Dmax} = 0.7$, $\psi_{Rmax} = 1.0$ h^{-1}; conjugation rates $\gamma_{Dmax} = 10^{-14}$ ml $\cdot$ CFU^{-1}h^{-1}, $\gamma_{Tmax} = 10^{-11}$ ml $\cdot$ CFU^{-1}h^{-1}; approximation factor $f = 10$.

measurement time point. If the initial population densities are manipulated, but the ratio of D:R is kept constant at 1:1 (Figure 2A), the T/D measure declines roughly proportional to the reduced initial population density. Instead, if the total population density is kept constant, but the relative ratios of recipient and donor densities are varied (Figure 2B), it becomes clear that the T/D measure declines roughly proportional to the change in initial recipient population density.

The interpretation of population density based measures such as T/D is therefore difficult, due to their sensitive dependence on initial population densities, donor to recipient ratios, and time of measurement. In experiments where the experimental condition affects the initial donor and recipient population densities or ratios, the measure T/D will confound this bias with any effect of the experimental condition on the conjugation rates themselves. More generally, this will also be the case for the other population density based measures.

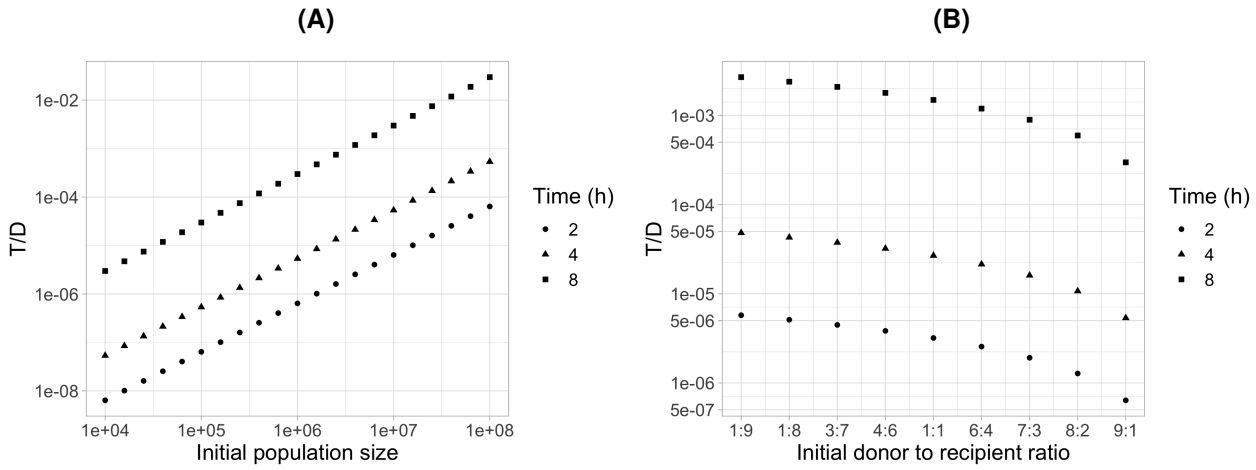

Figure 2: Impact of initial population density **(A)**, and donor-recipient ratio **(B)** on the T/D conjugation frequency estimate. The estimate varies over several orders of magnitude as a function of the initial population densities, relative population densities, and measurement time point. Parameters: initial resource $C_0 = 10^{14}$ μg/mL; growth rates $\psi_{Tmax} = \psi_{Dmax} = \psi_{Rmax} = 1.0$ h^{-1}; conjugation rates $\gamma_{T/Dmax} = 10^{-13}$ ml $\cdot$ CFU^{-1}h^{-1}. For panel **(A)**, the initial population densities are $D_0, R_0 \in [10^4, 10^8]$ CFU/mL. Recipient and donor populations are kept at the same density. For panel **(B)**, the ratio between initial population densities is $D_0 : R_0 \in [9:1, 1:9]$, with the total population density constant at 10^7 CFU/mL.

Extending the Simonsen method

We have seen that population-based measures are not robust to variation in (i) the time-duration of the assay (Fig. 1B), (ii) initial population densities (Fig. 2A), (iii) and donor to recipient ratios (Fig. 2B). The end-point method based on the Simonsen model (SM), which has been around for 30 years, is robust to these factors. However, this method is not applicable to populations with differing growth rates, nor differences in conjugation rates from donors and transconjugants. Thus, we extended the SM for differing growth and conjugation rates (see methods section), and derived an end-point formula for this new model (the ASM), which is easily computed on experimental data (see supplementary materials).

In deriving the ASM estimate, we make some assumptions about the relative size of different processes contributing to the overall dynamics of D, R and T populations. Some of these assumptions are also tacitly made in the SM estimate. Most prominently, this includes the assumptions that (i) the recipient population is not substantially reduced due to transformation to transconjugants, and (ii) no conjugation takes place in stationary phase. If the rates of conjugation from donors and transconjugants differ, both the SM and ASM further require that (iii) the populations were measured at a time where the dynamics are still dominated by conjugation events between donors and recipients rather than between transconjugants and recipients. When these assumptions are no longer valid, we expect the SM and ASM estimates for the donor conjugation rate to fail. By making these as-

sumptions explicit, we can derive the critical time t_{crit} beyond which the approximations break down (see the supplementary materials). Importantly, this critical time t_{crit} is the minimum of three different time points, reached when one of the approximations (i) or (iii) fails. Which of these time points is reached first, and thus which dictates the latest possible measurement time point, depends on the relative size of the growth rates ($\psi_{Dmax}, \psi_{Rmax}, \psi_{Tmax}$), conjugation rates ($\gamma_{Dmax}, \gamma_{Tmax}$), as well as the initial population densities (D_0, R_0, see supplementary materials).

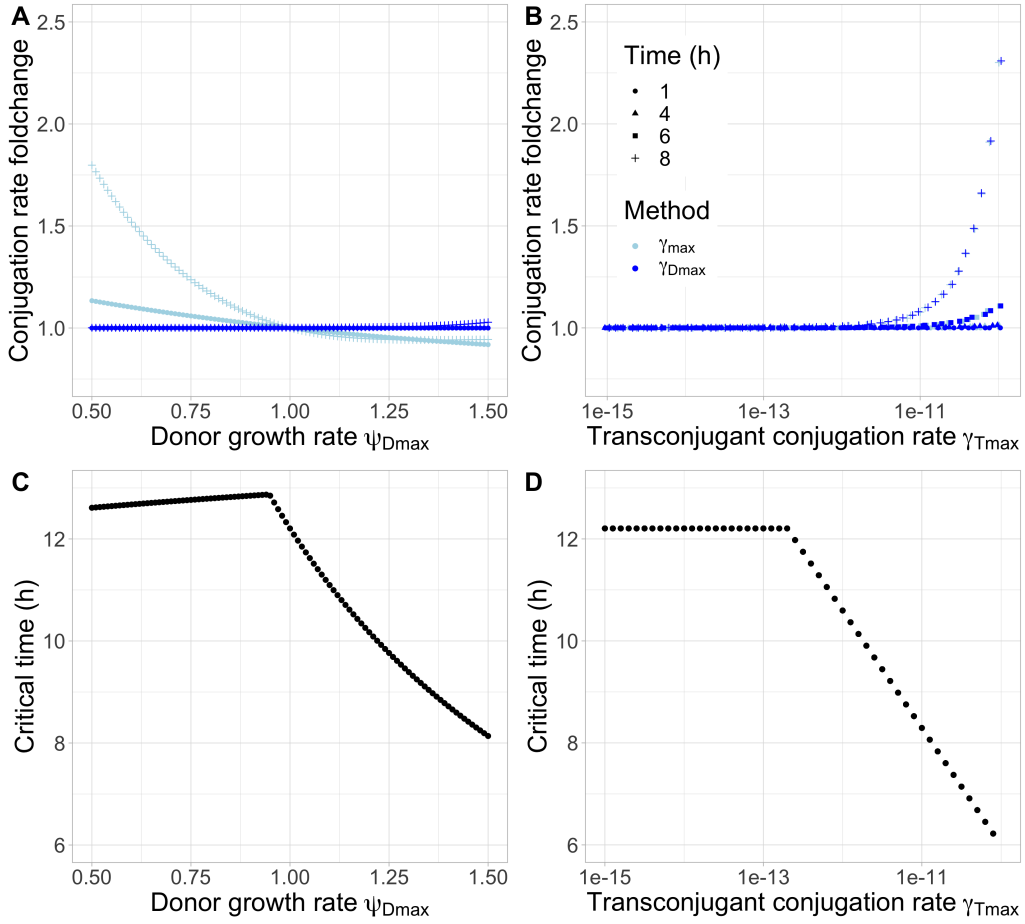

Figure 3: Panels **(A, B)** show the deviation of the estimated conjugation rate from the true value in the simulation, for different measurement time points, as a function of the donor growth rate **(A)** or transconjugant conjugation rate **(B)** where γ_{max} denotes the SM estimate and γ_{Dmax} the ASM estimate. Panels **(C, D)** show the corresponding critical time. Faster donor growth and transconjugant conjugation rates reduce the critical time **(C, D)**, which is mirrored by the greater deviation of the estimated conjugation rates from the true value, i.e. a fold change of 1, for later measurement time points **(A, B)**. For deviating growth rates, the SM always shows a minor estimation error **(A)**, whereas for deviating transconjugant conjugation rates both methods are correct within the critical time **(B)** (the methods are partially overlaid). Fold change is defined as the ratio between the estimated value and the true value. Parameters: initial population densities $R_0 = D_0 = 5 \cdot 10^6$ CFU/mL; initial resources $C_0 = 10^{14}$ μg/mL; growth rates $\psi_{Tmax} = \psi_{Rmax} = 1.0$ h^{-1}; conjugation rate $\gamma_{Dmax} = 10^{-13}$ ml $\cdot$ CFU^{-1}h^{-1}; approximation factor $f = 10$ are the same for all panels. For panels **(A, C)**, growth rate $\psi_{Dmax} \in [0.5, 1.5]$ h^{-1} and conjugation rate $\gamma_{Tmax} = 10^{-13}$ ml $\cdot$ CFU^{-1}h^{-1}; for panels **(B, D)**, growth rate $\psi_{Dmax} = 1.0$ h^{-1} and conjugation rate $\gamma_{Dmax} \in [10^{-15}, 10^{-10}]$ ml $\cdot$ CFU^{-1}h^{-1}.

We use simulated data to investigate whether the ASM estimate improves the conjugation rate esti-

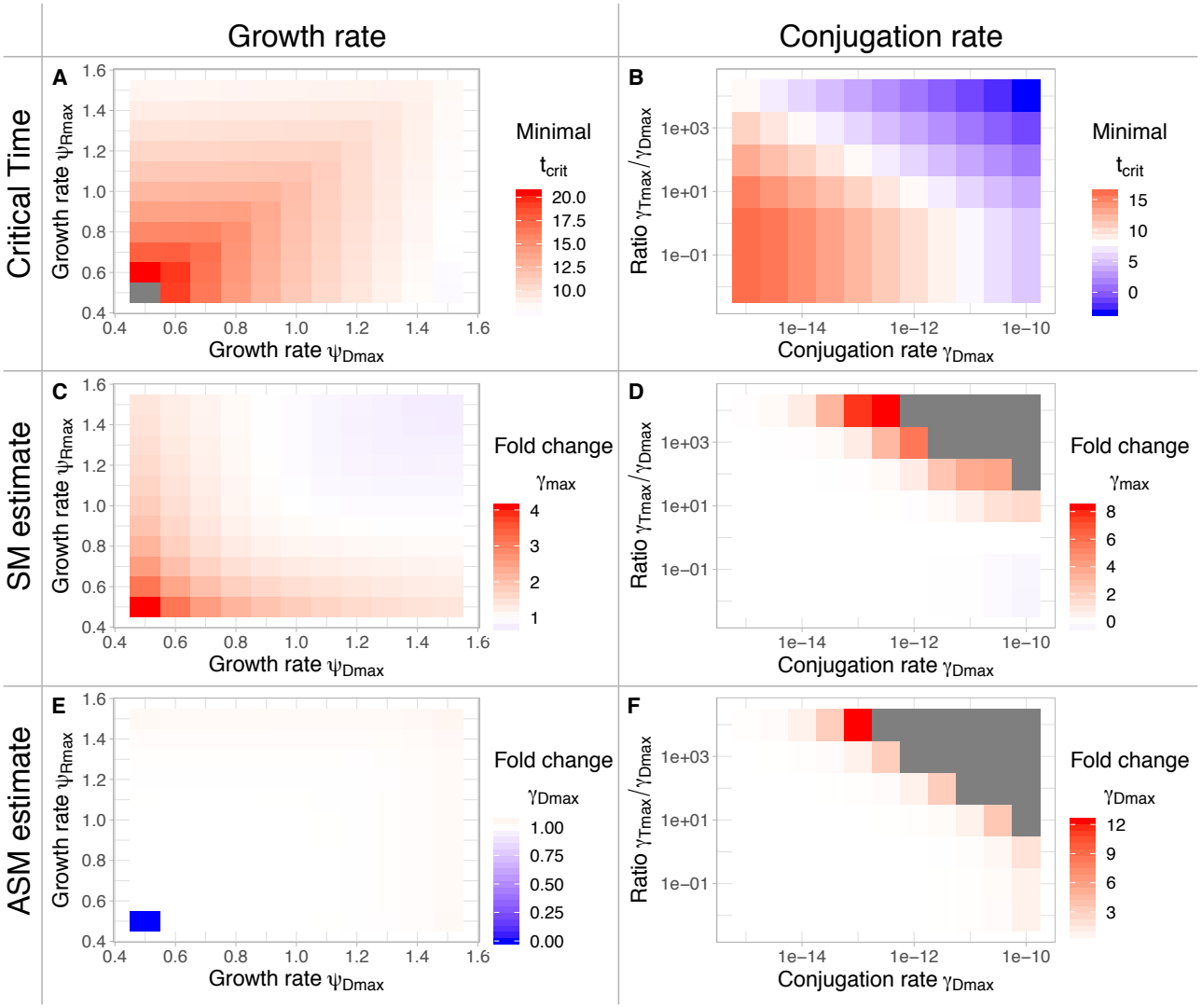

Figure 4: The critical time **(A, B)**, SM estimate γ_{max} **(C, D)**, and ASM estimate γ_{Dmax} **(E, F)**, as a function of the growth (First column: **(A, C, E)**) or conjugation rates (Second column: **(B, D, F)**). For the estimation **(C, D, E, F)** we assume the system is measured after 8 hours; the blue regions in panels **(A, B)** correspond to a critical time below this value. The ratio $\gamma_{Tmax}/\gamma_{Dmax}$ **(B, D, F)** indicates how much bigger the rate of conjugation from transconjugants is than that from donors. Fold change is defined as the ratio between the estimated value and the true value. Faster donor growth and transconjugant conjugation rates reduce the critical time **(A, B)**. For deviating growth rates, the SM always shows a minor estimation error **(C)**, whereas for deviating transconjugant conjugation rates both methods are correct within the critical time **(B, D, F)**. The ASM estimate is not valid when $2\psi_{Tmax} = \psi_{Rmax} = \psi_{Dmax}$, which leads to the zero value in the lower left corner of panels **(A, E)**. Both the SM and ASM result in numerical errors when measuring substantially above the critical time, upper right corner of panels **(D, F)**. Parameters: initial population densities $R_0 = D_0 = 5 \cdot 10^6$ CFU/mL; initial resources $C_0 = 10^{14}$ μg/mL; growth rate $\psi_{Tmax} = 1.0$ h^{-1}; approximation factor $f = 10$. For panels **(A, C, E)**, growth rates $\psi_{Dmax}, \psi_{Rmax} \in [0.5, 1.5]$ h^{-1} and conjugation rates $\gamma_{Dmax} = \gamma_{Tmax} = 10^{-13}$ ml$\cdot$CFU^{-1}h^{-1}. For panels **(B, D, F)**, growth rates $\psi_{Dmax} = \psi_{Rmax} = 1.0$ h^{-1} and conjugation rates $\gamma_{Dmax} \in [10^{-15}, 10^{-10}]$ ml $\cdot$ CFU^{-1}h^{-1}, $\gamma_{Tmax} \in [10^{-17}, 10^{-6}]$ ml $\cdot$ CFU^{-1}h^{-1}.

mate in the face of differing (i) growth, and (ii) conjugation rates. Here, we use the fold change, i.e. the ratio between the estimated value and the true value of the conjugation rate γ_{max}, to quantify the error made during estimation.

Growth As can be seen in Figures 3A and 4C, the SM estimate varies as a function of the donor and recipient population growth rate. The SM overestimates the conjugation rate if donor and/or recipients populations grow more slowly than the transconjugant population (lower left corner of Fig. 4C). If the transconjugants grow more slowly than D and/or R, the SM underestimates the conjugation rate (upper right corner of Fig. 4C). This is the case for all measurement time points, although the effect is exacerbated for measurements that are made after a longer conjugation time (Fig. 3A). In contrast, the new ASM estimate γ_{Dmax} is valid until the critical time t_{crit}, i.e. the time point for which the approximations of the model break down (Fig. 4E). The critical time window grows shorter as the absolute magnitude of the growth rates increases (Fig. 3C, 4A, and S1). Because the critical time is determined as the minimum of three different processes, all of which depend on the growth rates in different ways, the process dictating the critical time changes as a function of the growth rate. In Fig. 3C the limiting process is first the early onset of substantial conjugation from transconjugants (time t_{c1}, see supplementary materials) and then the substantial reduction of the recipient population due to conjugation events (time t_{c2}, see supplementary materials).

Conjugation rates If the rates of conjugation from donors and transconjugants differ, both the SM and the ASM estimates accurately estimate the donor to recipient conjugation rate, as long as D, R, T are measured sufficiently early (Fig. 4D/F). This is because the contribution of TRT conjugation events will be small as long as the transconjugant population is still small. For later times, the estimated SM conjugation rate γ_{max} will interpolate between γ_{Dmax} and γ_{Tmax}. The estimated time at which the approximations break down (t_{crit}) is the same for both methods (Fig. 4B). As can be seen in Figures 3B and 4D/F, this means that the magnitude of the misestimation of SM and ASM estimates depends strongly on the measurement time point. This shows that it is critically important not to measure too late.

4 Protocol

These theoretical considerations have led us to propose the following protocol to perform conjugation assays. In its most complete form the protocol requires two conjugation experiments: a first one starting from a $D+R$ mixed culture, and then a second one with $T+R$. As pointed out in the previous section, it is important that the population densities of D, R and T are measured before the critical time is reached. Strictly speaking, this critical time can only be determined after both conjugation experiments are completed, as they require an estimate of both conjugation rates ($\gamma_{Dmax}, \gamma_{Tmax}$),

as well as all growth rates ($\psi_{Dmax}, \psi_{Rmax}, \psi_{Tmax}$, see supplementary materials). To optimise the chance of measuring below the critical time, we recommend to measure as soon as a measurable number of transconjugants has been formed. Note, if one can assume that the difference between γ_D and γ_T is negligible, then the second conjugation experiment with $T + R$ is not necessary.

- Run 1st experiment with D and R:
  - Grow overnight cultures of D and R.
  - Incubate cultures of D and R in isolation and as a mixed culture of $D + R$. Measure the growth rates of all cultures in exponential phase. This yields estimates for the growth rates ψ_{Dmax} and ψ_{Rmax} from the single cultures, as well as ψ_{max} from the mixed culture.
  - Plate the mixed culture on selective plates at a time point, t_1, to estimate the population densities of D, R and T. This time point should be early enough, such that there is a high chance that it is below the critical time $t_{crit,1}$ for the 1st experiment.
  - Calculate the ASM estimate for the conjugation rate from donors γ_{Dmax}.
  - In case you are considering not to perform the 2nd conjugation experiment, you can use the Shiny app or R package to determine how sensitively the estimate of the conjugation rate γ_{Dmax} depends on the presumed values of the conjugation rate from transconjugants γ_{Tmax}.
- Run 2nd experiment with T and R':
  - Isolate single transconjugant clones T from the 1st experiment, to use as plasmid donors in the 2nd experiment. Either these clones or the recipients used in this 2nd experiment need to be provided with an additional selective marker such that the transconjugants of the 2nd experiment (T') can be distinguished from those of the 1st experiment (T).
  - Grow overnight cultures of T and R'.
  - Incubate cultures of T and R' in isolation and as a mixed culture of $T + R'$. Measure the growth rates of all cultures in exponential phase. This yields estimates for the growth rates ψ_{Tmax} from the single cultures, as well as ψ_{max} from the mixed culture.
  - Plate the mixed culture on selective plates at a time point, t_2, to estimate the population densities of T, R' and T'. This time point should be early enough, such that there is a high chance that it is below the critical time $t_{crit,2}$ for the 2nd experiment.

- Estimate the conjugation rate from transconjugants γ_{Tmax}.
- Check whether $t_2 < t_{crit,2}$ for the 2nd experiment.
- If the 2nd experiment is within the critical time, check whether $t_1 < t_{crit,1}$ for the 1st experiment.
- If either t_1 or t_2 are too large, the experiments will need to be repeated, choosing times smaller than t_{crit}.

5 Tools for the scientific community

We present a Shiny app (a beta-version is currently available under `https://ibz-shiny.ethz.ch/jhuisman/conjugator/`), which allows researchers to (i) simulate bacterial population dynamics with conjugation (ii) upload their own data, calculate conjugation rates, and check whether a given experiment was measured within the critical time. An R package will also be made available soon.

6 Discussion

There is no gold standard to determine and report conjugation rates, and this has complicated the comparison of experimental values obtained by different research groups or under different conditions [10].

We extended the Simonsen model for conjugation rate estimation to include the effects of differential population growth and conjugation rates from donors and transconjugants. We derived a new endpoint method to estimate conjugation rates under this model, as well as expressions for the critical time after which this approximation breaks down.

A clear conclusion of this work is that one should measure the outcome of conjugation assays early, before the dynamics become dominated by conjugation from transconjugants. Our critical time gives an indication of how early this should be.

If the donor, recipient, or transconjugant populations differ in their growth rates, the Simonsen model makes a minor estimation error that is corrected by using our new ASM estimate. When the conjugation rate from transconjugants differs substantially from the donor conjugation rate, both methods estimate a correct conjugation rate up to the critical time. Overall, we find that bacteria with large

growth rate differences, high absolute growth rates, and high absolute conjugation rates are most likely to lead to problems in conjugation rate estimation, as these factors speed up the population dynamics and reduce the critical time.

Several caveats remain for both the SM and the ASM. First, these models are in principle not suitable for application to mating assays on solid surfaces, as they assume well-mixed conjugating populations. However, the conjugation rates in high-density, well mixed surface mating experiments are comparable to liquid mating, provided they are measured sufficiently early [13]. Second, the ASM assumes that the growth rates in monoculture are predictive for the same strains in mating populations, and thus disregards competitive effects. Last, neither method includes segregational loss. These concerns could be addressed by constructing a more complex conjugation and growth model and fit it to this data [15, 22, 29]. The reason we have chosen an end-point method instead is to minimise the experimental effort needed, at only a minor cost to the precision of the estimate.

We propose to settle on one method to describe conjugation proficiency [14]. Ideally, such a measure would allow comparison across experimental conditions, and to parametrise mechanistic models used to explain and predict plasmid dynamics.

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9 Supplementary materials

9.1 End-point method for the approximate extended Simonsen model

We aim to derive a simple end-point method to estimate the conjugation rate from donors, analogous to the SM estimate. To do so, we start from the equations for the approximate extended Simonsen model (ASM; eqs. 2.10-2.12 in the Methods section). Assuming that (i) initially the recipient population dynamics are dominated by growth, and that (ii) the transconjugant population is not yet dominated by conjugation from transconjugants, i.e.

$$(i) \qquad \psi_{Rmax}R >> \gamma_{Tmax}TR + \gamma_{Dmax}DR \tag{9.1}$$

$$(ii) \qquad \psi_{Tmax}T + \gamma_{Dmax}DR >> \gamma_{Tmax}TR \tag{9.2}$$

We obtain a simplified set of equations given by:

$$\dot{D} = \psi_{Dmax}D \tag{9.3}$$

$$\dot{R} = \psi_{Rmax}R \tag{9.4}$$

$$\dot{T} = \psi_{Tmax}T + \gamma_{Dmax}DR \tag{9.5}$$

We solve this for initial conditions corresponding to an 'invasion from rare' scenario, i.e. $D(0) = D_0, R(0) = R_0$ and $T(0) = 0$, and get the solution:

$$D(t) = D_0 e^{\psi_{Dmax}t} \tag{9.6}$$

$$R(t) = R_0 e^{\psi_{Rmax}t} \tag{9.7}$$

$$T(t) = \gamma_{Dmax}D_0R_0\frac{e^{(\psi_{Dmax}+\psi_{Rmax})t} - e^{\psi_{Tmax}t}}{\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}} \tag{9.8}$$

Conjugation rate γ_D

This solution for T (eq. 9.8) contains the conjugation rate γ_{Dmax}. By rearranging the terms, and using equations 9.6 and 9.7 to substitute $D(t)R(t) = D_0R_0e^{(\psi_{Dmax}+\psi_{Rmax})t}$, we obtain an estimate of the conjugation rate γ_{Dmax} at a time point t:

$$\gamma_{Dmax} = (\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}) \frac{T(t)}{D(t)R(t) - D_0 R_0 e^{\psi_{Tmax} t}} \tag{9.9}$$

This expression (the ASM estimate) can be used instead of the SM estimate as long as the approximate solutions (eqs. 9.6 - 9.8) are good approximations to the full ODE (eqs. 2.10 - 2.12).

Critical time t_{crit}

In deriving the ASM estimate, we made some approximations (eqs. 9.1 and 9.2) about the relative size of different processes contributing to the overall dynamics of D, R and T populations (leading to eqs. 9.6 - 9.8). When these approximations are no longer valid, the ASM estimate for γ_{Dmax} (eq. 9.9) fails. However, we can calculate the 'critical time' beyond which the approximations no longer hold.

First, the equation for $T(t)$ (eq. 9.8) fails to approximate the solution of the full ODE (eq. 2.12) once conjugation from transconjugants is substantial, i.e. once $\gamma_{Tmax} T(t) R(t) \approx \psi_{Tmax} T(t) + \gamma_{Dmax} D(t) R(t)$. If we specify a factor f by which the left hand side (conjugation from transconjugants) should be smaller than the right hand side (clonal growth of transconjugants and conjugation from donors), we obtain an equation for the time t_{c1} when the approximation will be violated:

$$f \gamma_{Tmax} R(t_{c1}) = \psi_{Tmax} + \gamma_{Dmax} \frac{D(t_{c1}) R(t_{c1})}{T(t_{c1})} \tag{9.10}$$

Here we already divided by $T(t)$ on both sides. For the last term of this equation, we can substitute our approximation of γ_{Dmax} (eq. 9.9) to obtain:

$$\gamma_{Dmax} \frac{D(t)R(t)}{T(t)} = (\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}) \frac{D(t)R(t)}{D(t)R(t) - D_0 R_0 e^{\psi_{Tmax} t}} \tag{9.11}$$

$$= (\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}) \frac{e^{(\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax})t}}{e^{(\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax})t} - 1} \tag{9.12}$$

$$\approx \psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax} \tag{9.13}$$

where we first substituted the definitions of $D(t)$ and $R(t)$, and the last equality holds for $t >> 1/(\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax})$, i.e. at times t substantially larger than the bacterial doubling time.

Substituting this expression (eq. 9.13) into equation 9.10 we get:

$$f\gamma_{Tmax}R_0e^{\psi_{Rmax}t_{c1}} = \psi_{Tmax} + (\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}) \tag{9.14}$$

and thus, for the first critical time:

$$t_{c1} = \log[\frac{\psi_{Dmax} + \psi_{Rmax}}{f\gamma_{Tmax}R_0}]/\psi_{Rmax} \tag{9.15}$$

Second, $R(t)$ (eq. 9.7) fails to approximate the solution of the full ODE once the recipient population dynamics are no longer dominated by growth, i.e. $\psi_{Rmax}R(t) \approx \gamma_{Dmax}D(t)R(t) + \gamma_{Tmax}T(t)R(t)$. To simplify this we break the approximation down into two parts: (i) $\psi_{Rmax} \approx \gamma_{Dmax}D(t)$ and (ii) $\psi_{Rmax} \approx \gamma_{Tmax}T(t)$. Substituting the above expressions for D and T (eqs. 9.6-9.8) into these equations we get the following:

$$\psi_{Rmax} = f\gamma_{Dmax}D_0e^{\psi_{Dmax}t_{c2}} \tag{9.16}$$

$$\psi_{Rmax} = f\gamma_{Tmax}\gamma_{Dmax}D_0R_0\frac{e^{(\psi_{Dmax}+\psi_{Rmax})t_{c3}} - e^{\psi_{Tmax}t_{c3}}}{\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}} \tag{9.17}$$

For the second equation (eq 9.17) we again assume that the time t is substantially larger than the doubling time of the bacteria, i.e. $t >> 1/(\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax})$, to simplify it to:

$$\psi_{Rmax} = f\gamma_{Tmax}\gamma_{Dmax}D_0R_0\frac{e^{(\psi_{Dmax}+\psi_{Rmax})t_{c3}}}{\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}} \tag{9.18}$$

By solving equations 9.16 and 9.18 for time, we obtain the two further critical times:

$$t_{c2} = \log(\frac{\psi_{Rmax}}{f\gamma_{Dmax}D_0})/\psi_{Dmax} \tag{9.19}$$

$$t_{c3} = \log(\frac{\psi_{Rmax}(\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax})}{f\gamma_{Dmax}\gamma_{Tmax}D_0R_0})/(\psi_{Dmax} + \psi_{Rmax}) \tag{9.20}$$

The ASM estimate will lose its validity as soon as one of the three critical times is reached. Depending

on the parameters (the relative sizes of growth and conjugation rates, as well as initial population densities) this could be any one of $t_{c1} - t_{c3}$. With 'the' critical time t_{crit}, we thus refer to the minimum $t_{crit} = \min(t_{c1}, t_{c2}, t_{c3})$ of these three time points:

$$t_{c1} = \log[\frac{\psi_{Dmax} + \psi_{Rmax}}{f\gamma_{Tmax}R_0}]/\psi_{Rmax} \tag{9.21}$$

$$t_{c2} = \log(\frac{\psi_{Rmax}}{f\gamma_{Dmax}D_0})/\psi_{Dmax} \tag{9.22}$$

$$t_{c3} = \log(\frac{\psi_{Rmax}(\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax})}{f\gamma_{Dmax}\gamma_{Tmax}D_0R_0})/(\psi_{Dmax} + \psi_{Rmax}) \tag{9.23}$$

9.2 Stationary phase time

Stationary phase is reached at the time t_{stat} when all initial resources $C(t = 0) = C_0$ have been consumed by the growing bacteria, and converted into biomass; i.e.

$$C_0 = e\,(N(t_{stat}) - N(0)) \tag{9.24}$$

In a general case, $N(t) = D(t) + R(t) + T(t)$ depends on the growth rate of all three populations, and the rate at which recipients are turned into transconjugants. If we substitute our earlier approximations for D(t), R(t) and T(t) we get:

$$N(t) = D_0e^{\psi_{Dmax}t} + R_0e^{\psi_{Rmax}t} - \gamma_{Dmax}D_0R_0\frac{e^{(\psi_{Dmax}+\psi_{Rmax})t} - e^{\psi_{Tmax}t}}{\psi_{Dmax} + \psi_{Rmax} - \psi_{Tmax}} \tag{9.25}$$

When all populations grow at the same rate ψ_{Xmax}, any transformation of recipients into transconjugants does not affect the total population growth of N. If we assume simple exponential growth (as opposed to e.g. Monod dynamics), $N(t)$ will be given by:

$$N(t) = N_0e^{\psi_{Xmax}t} = (R_0 + D_0)e^{\psi_{Xmax}t} \tag{9.26}$$

With this equation 9.24 becomes:

$$C_0 = eN_0\left(e^{\psi_{Xmax}t} - 1\right) \tag{9.27}$$

$$\Rightarrow t_{stat} = \frac{1}{\psi_{Xmax}} \ln\left(1 + \frac{C_0}{eN_0}\right) \tag{9.28}$$

where e is in μg per CFU. Population densities N, D, T, R, N_0 in CFU/mL. Resource C in μg per mL. Growth rate ψ_{Xmax} is per hour.

In cases where we observe the mating population, we can simply replace ψ_{Xmax} by the growth rate of that mixed population (ψ_{max} from the Simonsen model). If one were to include Monod like growth dynamics, this would slow down growth at high population densities/ as the resource is becoming depleted. As a result, the start of stationary phase t_{stat} would be slightly delayed.

9.3 The impact of higher conjugation rates on conjugation rate estimation

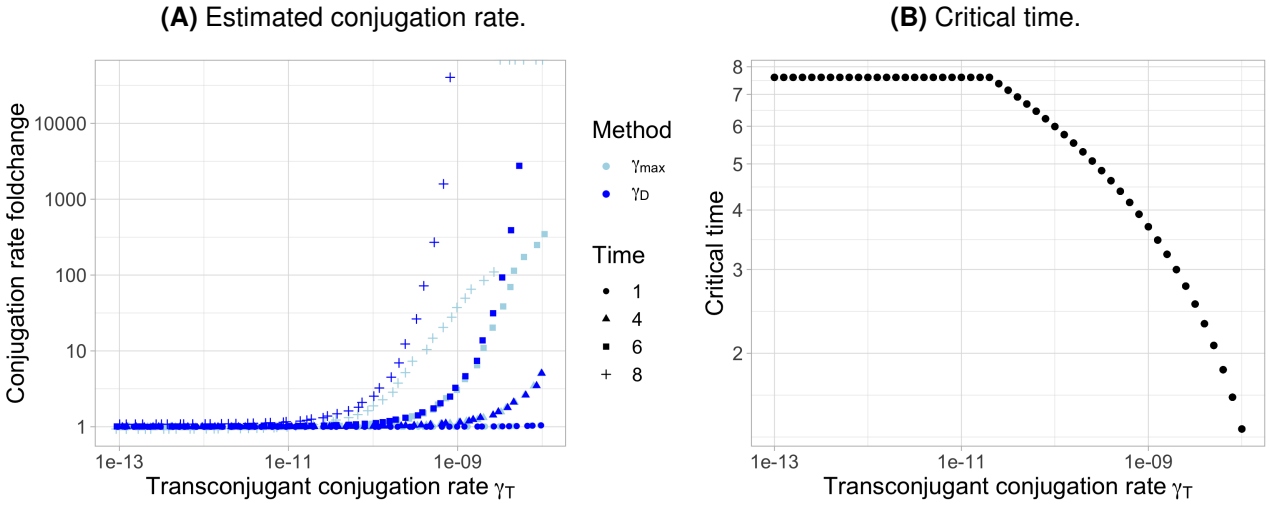

Figure S1: Panel **(A)** shows the ratio between the estimated conjugation rate and the true value in the simulation, for different measurement time points, as a function of the donor growth rate (γ_{max} denotes the SM estimate, γ_{Dmax} the ASM estimate). Panel **(B)** shows the corresponding critical time. The parameters mimic those in Fig. 3 in the main text, except that the conjugation rates are higher, and thus the temporal dynamics are faster. Initial population densities $R_0 = D_0 = 5 \cdot 10^6$ CFU/mL; initial resource concentration $C_0 = 10^{14}$ μg/mL; growth rates $\psi_{Tmax} = \psi_{Dmax} = \psi_{Rmax} = 1.0$ h^{-1}; conjugation rate $\gamma_{Dmax} = 10^{-11}$ ml $\cdot$ CFU^{-1}h^{-1}; approximation factor $f = 10$.