Understanding microbial cooperation

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Supplemental Information

Contents

1 Introduction

The supplemental material elaborates on some of the comments made in the main text, but is in many ways distinct and makes some novel points. To increase readability, we omit some of the proofs and refer the reader to the original papers that we cite for more detailed treatments. Each section is largely autonomous and so the reader should feel free to skip to whichever areas are of particular interest.

2 Hamilton's rule

2.1 Relatedness and assortment

In the main text, we introduced relatedness as a linear regression coefficient, but because this is not necessarily very intuitive, we will include some simpler definitions that apply if we are only considering pairwise interactions between two competing strains with simple population structure and global competition. First, r can represent the extent of kin discrimination, namely that a proportion r of all interactions are with known relatives and $1-r$ are with a random member of the population (Grafen, 1979). This definition can be useful when active discrimination takes place and it combines nicely with many game theory approaches.

Additionally, r can be seen as the difference in probability for a cooperator to interact with a cooperator versus a defector interacting with a cooperator (van Veelen, 2009). To illustrate the equivalence between the two definitions, let us find the probability for a cooperator to interact with a cooperator, $\mathbb{P}(C|C)$, and for a defector to interact with a cooperator, $\mathbb{P}(C|D)$, using the previous definition of relatedness as an extent of kin discrimination:

$$\mathbb{P}(C|C) = 1 \cdot r + (1-r) \cdot p \quad (1)$$

$$\mathbb{P}(C|D) = 0 \cdot r + (1-r) \cdot p = (1-r) \cdot p \quad (2)$$

where p is the proportion of cooperators in the population. It can now be seen that $\mathbb{P}(C|C) - \mathbb{P}(C|D) = r$ as claimed (Figure 1A).

A third definition is Grafen's famous geometric view of relatedness (1985, Figure 1B). The relatedness coefficient becomes how close a recipient's genotype or phenotype is to your own relative to the population average. As physicists, we see a connection between this definition and a center of mass problem. The question then becomes: if I decrease my mass by c to increase the recipient's mass by b, will the population average increase towards my phenotype or decrease away from my phenotype (i.e., is $rb-c > 0$?). Relatedness in this concept is unbounded and can be negative, which is fine.

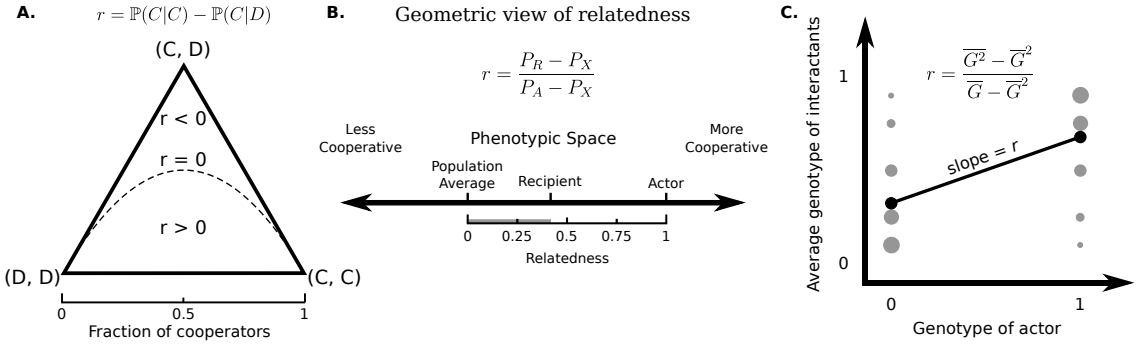

Figure 1: There are many equivalent ways to visualize relatedness, each with their relative merit. (**A**) Relatedness can be seen as the amount of homogeneous interactions (cooperators, C, with other cooperators and defectors, D, with other defectors) relative to number of heterogeneous interactions (cooperators with defectors). As interactions become more assorted than expected by chance, relatedness is positive. On the other hand, if cooperator-defector interactions (C, D) are more common than by chance, relatedness is negative (adapted from van Veelen (2011)). (**B**) The geometric view of relatedness provides an intuitive and visual definition of relatedness. P_A, P_R, and P_X are the phenotype of the Actor and the average phenotype of the Recipient and population, respectively. A geometric view of genetic relatedness is also possible and similar, but a phenotypic view is more intuitive if organisms are haploid and if phenotype is a simple function of genotype. Note the apparent similarity to equation (4). (**C**) When individuals are partitioned into groups, relatedness is proportional to the variance in the fraction of cooperators per group, G here. Relatedness can also be seen as the difference in the average number of cooperators a cooperator interacts with versus a defector (black dots).

If individuals are partitioned into groups of equal sizes and every individual in a group mutually interacts, then relatedness becomes:

$$r = \frac{\mathrm{Cov}(G_X, G_Y)}{\mathrm{Cov}(G_X, G_X)} = \frac{\mathrm{Var}(G_g)}{\mathrm{Var}(G_X)} = \frac{\overline{G_g^2} - \overline{G_g}^2}{\overline{G_g} - \overline{G_g}^2} \tag{3}$$

where G_g is the average genotype of a group and G_X is the genotype of an individual (Figure 1C, (Pepper, 2000)). This ratio of variances becomes important when thinking in terms of group selection; if every group has the same composition of cooperators, then $\mathrm{Var}(G_g) = 0$ and relatedness is 0. On the other hand, if every group is either all cooperators or all defectors, then $\mathrm{Var}(G_g) = \mathrm{Var}(G_X)$ and relatedness is 1. Relatedness can then be thought of as the proportion of variance in the trait that is due to the variance between groups (Breden, 1990).

Dividing numerator and denominator of equation (3) by $\overline{G_g}$ gives:

$$r = \frac{\frac{\overline{G_g^2}}{\overline{G_g}} - \overline{G_g}}{1 - \overline{G_g}} = \frac{\mathbb{P}_{A\to R} - \mathbb{P}_{A\to P}}{\mathbb{P}_{A\to A} - \mathbb{P}_{A\to P}} \tag{4}$$

where $\mathbb{P}_{A\to R}$ is, formally, the probability that a gene drawn at random from the Actor at the focal loci (e.g. the gene coding for the cooperative behavior) is identical in state to a gene drawn at random from the Recipient at the focal loci. Similarly, $\mathbb{P}_{A\to P}$ compares the Actor to a member of the population drawn at random and $\mathbb{P}_{A\to A}$ compares the Actor to itself ($\mathbb{P}_{A\to A} = 1$ for haploids). In the case of a rare gene in a large population, $\mathbb{P}_{A\to P} \to 0$, and $r \to \frac{\mathbb{P}_{A\to R}}{\mathbb{P}_{A\to A}}$ which gives the classic result that r is one half for siblings and one eighth for cousins (in the absence of inbreeding). Note that equation (4) is more general than how it

was derived here and is the value of relatedness often quoted in the inclusive fitness literature (West et al., 2006). It should be noted here that in more complicated theoretical models, relatedness is often defined such that Hamilton's rule works. Some have argued that this casts serious doubt on the fundamental nature of inclusive fitness (Nowak et al., 2010), especially since Tarnita et al. (2009) recently showed that a rule linear in b and c can always be written because of the linearity imposed by weak selection and not necessarily anything fundamental about inclusive fitness (see section 4).

While there are many ways to correctly measure and conceptualize the assortment of similar individuals, there are also many wrong ways to do it. Because Hamilton's rule only asks if an allele will increase in frequency (and not necessarily in number), any definition that does not take into account the overall population will be false. Therefore, relatedness is not the percent of genome shared, genetic distance, or any extent of similarity between two isolated individuals in a larger population. Also, because horizontal gene transfer is commonplace between microbes and selection is strong, phylogenetic distance or any other indirect genetic measure is likely to be inaccurate. These errors are often very easy to make and models are rarely made to check the validity of naïve guesses of relatedness, which becomes very dangerous when uninformed scientists incorrectly try to apply Hamilton's rule to gain false intuitions about the evolution of cooperation.

2.2 Queller's synergistic term

Queller has made significant progress generalizing Hamilton's rule and fixing some cases where it is less intuitive (1984; 1985; 1992; 1994). One of these cases is when an interaction cannot be simply described by a cooperator paying a fixed cost, c, for another individual to gain a fixed benefit, b. For situations where cooperators gain d more (or $-d$ less) from cooperation than defectors do, a modified Hamilton's rule is often more appropriate:

$$\frac{\mathrm{Cov}(G_X, G_Y)}{\mathrm{Cov}(G_X, G_X)}\beta_{WG_Y} + \beta_{WG_X} + \frac{\mathrm{Cov}(G_X, G_X G_Y)}{\mathrm{Cov}(G_X, G_X)}\beta_{W,G_X G_Y} = rb - c + md > 0 \tag{5}$$

where, roughly, m is how often cooperators interact with each other and d is a measure of how much more cooperators benefit from interacting with cooperators than do defectors (Queller, 1985). In many ways, this approach is similar to taking a regression of cooperator fitness as a function of social neighborhood separately from the regression of defector fitness as a function of social neighborhood. The slope of the defector's regression would be b and the cooperator's slope would be $b + d$ with a y-intercept c below the defector's y-intercept. While this goes a step further in acknowledging possible synergies and non-linearities, simply changing the slope of one of the regressions still cannot capture the full picture. With that said, there may be cases where this is more intuitive and predictive than Hamilton's rule.

2.3 Vectorized generalization

One of the major disadvantages of the linear regression forms of Hamilton's rule is that if any nonlinearities occur, then b and c change with the population structure, which makes disentangling fitness effects and r impossible. To solve this problem, smith et al. (2010) recently suggested an alternative formulation:

$$\mathbf{r} \cdot \mathbf{b} - c + \mathbf{m} \cdot \mathbf{d} > 0 \tag{6}$$

where the boldface represents that these quantities are now vectors. $\mathbf{b}$ and $\mathbf{d}$ are found by fitting the fitness of defectors and cooperators versus the frequency of cooperator interactants with an appropriate (non-linear) regression. The coefficients of the Taylor series of the fit to defector fitness are the elements of the vector $\mathbf{b}$ and the difference between the Taylor series for cooperators and defectors are the elements of $\mathbf{d}$. Again, c is the difference in y-intercept of the two fits. In this case, $\mathbf{m}$ is a vector of moments of the distribution of the frequency of cooperators in the social neighborhood of cooperators. The difference in the cooperator moment vector and the defector moment vector is $\mathbf{r}$. Thus, the first element of the relatedness vector is the difference of the first moment (average) for cooperators and defectors and is equivalent to $r = \mathbb{P}(C|C) - \mathbb{P}(C|D)$ given above. Therefore, if everything is linear, then $\mathbf{b}$ only has the first element (linear slope) and $\mathbf{d} = 0$, so (6) reduces to $rb - c > 0$.

The biggest advantage of smith et al.'s generalization is that the cost and benefit vectors are constant and do not change under different population structures. Also, it rightly places many of the non-linearities of the interaction in $\mathbf{r}$ rather than b and c. For example, two populations may have the same level of traditional

relatedness, but because of other differences in population structure (e.g., higher order relatedness), the linear regression formulations of b and c may be drastically different between the two populations, which is not the case here. Also, this way of measuring benefits makes it very obvious just how non-linear a system may be.

Unfortunately, smith et al.'s generalization does have some limitations. For example, it cannot take into account density or time dependence as written and is still inappropriate when multiple strains are competing. It is also unclear how to measure population structure for arbitrary environments like an agar plate where interaction strengths decrease with distance. Similar to the Price equation, the formula is still not a model and is no more useful that adding up fitnesses if the measured quantities are left unstudied. Also, the exact interpretation of higher order benefit and relatedness terms can be less intuitive—but perhaps a more accurate view of evolution and cooperation—than traditional Hamilton's rule. There also still exists plenty of room for error; for example, smith et al. used log-transformed data to fit their curves, which will cause the residuals to be correlated with the independent variables of the regression when the fitness is brought back to a linear scale, invalidating an assumption used in deriving their rule. We see smith et al.'s reformulation as a great step forward in acknowledging the limitations of traditional Hamilton's rule and the importance of non-linearities in microbial interactions and we would strongly recommend a close reading of their paper and supplemental online material.

3 Empirical studies revisited

3.1 Hamilton's rule is easy to mess up

Kümmerli et al. (2010) tried to apply Hamilton's rule to a simplified microbial model involving the cooperative production of iron-scavenging siderophores in *Pseudomonas aeruginosa*. They competed siderophore producing cooperators against siderophore negative defectors in 18 subpopulations (test tubes). Each test tube either had all cooperators, all defectors, or a 50-50 mix of cooperators and defectors. To find the relevant b and c for Hamilton's rule, they measured the number of colony forming units (CFU) at the end of 24 hours of growth for each strain in each condition. The measured quantities are thus the average fitness of cooperators and defectors in pure cultures and in the 50-50 mix ($W_{C,pure}$, $W_{D,pure}$, $W_{C,mix}$, and $W_{D,mix}$, respectively; note, we use a slightly different notation than Kümmerli et al.). Because these measured fitnesses don't perfectly fit a simple linear model, the only way to apply Hamilton's rule is by a linear regression analysis or another method that can accommodate non-linearities (e.g. including a synergy term and accounting for the proportion of cooperators in the population; Queller, 1985; Wenseleers et al., 2009). Unfortunately, the authors use the unmodified inequality, $RB - C > 0$, giving an incorrect result.

In their paper, $B = W_{C,pure} - W_{D,pure}$ and $C = \frac{1}{2}(W_{D,mix} - W_{C,mix})$. Even if the interaction followed a perfectly linear "tragedy of the commons" model where each cooperator pays a cost c to give the group a benefit of b, their B would be equal to $b - c$ and their C would be $\frac{c}{2}$, which are not Hamilton's b and c. The authors then use the fact that $RB - C$ is greater than 0 in some cases and less than 0 in other cases to predict and justify that cooperators are favored in the first case and disfavored in the second. Unfortunately, because their B and C are not the b and c of Hamilton's rule, this result is inaccurate. Note that this error may have been prevented if they graphed the data and were more explicit with their model and calculations (no formal justification for their B or C is given in the paper). Also, it was evident from their measurements that their B and C were not predictive because $B < C$ for one of their studies, which would mean that even if the population is fully assorted (i.e., cooperators interact only with cooperators and defectors interact only with defectors, $r = 1$), then cooperation could not evolve. This prediction is false because pure cooperator cultures grew better than pure defector cultures ($W_{C,pure} > W_{D,pure}$).

3.2 Multilevel selection under the false guise of Hamilton's rule

Chuang et al. (2010) used a standard model from multilevel selection, but ultimately confuse it with Hamilton's rule. This is particularly important because knowledge of the exact definition of different formulas and parameters is crucial for accurate understanding, communication, and application. While the two formulations are similar, and Chuang et al. do end up with an inequality of the form $rb - c > 0$, their b and r are not the benefit and relatedness coefficients from Hamilton's rule. To illustrate the difference, let us go through their derivation. In their model, they have several subpopulations each with a distinct proportion of cooperators, G_g, and average fitness, W_g. Using the Price equation, they have:

$$\overline{W}\Delta\overline{G} = \mathrm{Cov}(W_g, G_g) + \overline{W_g \Delta G_g} \tag{7}$$

where $\overline{W_g \Delta G_g}$ now describes the change in the proportion of cooperators in a group. However, $\overline{W_g \Delta G_g}$ looks like the left side of the equation, and we can iterate the Price equation once more to get:

$$\overline{W}\Delta\overline{G} = \mathrm{Cov}(W_g, G_g) + \overline{\mathrm{Cov}_g(W_i, G_i)} + \overline{\overline{W_i}\Delta G_i} = \mathrm{Cov}(W_g, G_g) + \overline{\mathrm{Cov}_g(W_i, G_i)} \tag{8}$$

where W_i and G_i represent the fitness and genotype of an individual and the last equality is justified in the limit of low mutation rate. Equation (8) is a standard equation in multilevel selection theory where the first covariance describes the between-group benefit of being a cooperator and the second covariance captures the average within-group disadvantage of being a cooperator and is generally negative. If the between-group advantage is greater than the within-group disadvantage, then $\Delta\overline{G}$ will be positive and cooperation will spread.

The authors now deviate from the general equation above and assume that the within-group disadvantage is the same in every group. This allows them to remove the linear regression coefficient from the covariance out of the average:

$$\overline{W}\Delta\overline{G} = \beta_{W_g G_g}\mathrm{Var}(G_g) + \overline{\beta_{g,W_i G_i}\mathrm{Var}_g(G_i)} = \beta_{W_g G_g}\mathrm{Var}(G_g) + \beta_{g,W_i G_i}\overline{\mathrm{Var}_g(G_i)} \tag{9}$$

This last simplification is only justified when the cost of being a cooperator is constant, which is in general not true. Now, after dividing by $\overline{\mathrm{Var}(G_i)}$, we see that cooperators spread ($\Delta\overline{G} > 0$) when:

$$\beta_{W_g G_g}\frac{\mathrm{Var}(G_g)}{\overline{\mathrm{Var}_g(G_i)}} + \beta_{g,W_i G_i} > 0. \tag{10}$$

The authors now rename $\beta_{W_g G_g}$ as B, $\frac{\mathrm{Var}(G_g)}{\overline{\mathrm{Var}_g(G_i)}}$ as R and $\beta_{g,W_i G_i}$ as C, claiming that this is Hamilton's rule, but their R and B are different than Hamilton's r and b. That $B \neq b$ can be seen by assuming everything is linear, in which case each cooperator increases the group fitness by $b - c$, therefore $B = \beta_{W_g G_g} = b - c \neq b$. Also, their R is unbounded and approaches ∞ as the population becomes fully assorted because $\overline{\mathrm{Var}_g(G_i)} \to 0$. It is also clear after comparing R to r in equation (3):

$$R = \frac{\overline{G_g^2} - \overline{G_g}^2}{\overline{G_g} - \overline{G_g^2}} \neq \frac{\overline{G_g^2} - \overline{G_g}^2}{\overline{G_g} - \overline{G_g}^2} = r. \tag{11}$$

In fact, $R = \frac{r}{1-r}$. With all this said, the results of their experiment are still valid because they probably actually used equation (8). Also, their criticisms of Hamilton's rule (the lack of prediction power and intuition behind the ever-changing linear regressions) are still well-founded because their formulation used the Price equation and is similar. While $rb - c > 0$ is probably one of the most well known rules in biology, very few people actually know and use the true definition of r, b, and c.

3.3 "Fitness" is ambiguous

The term "fitness" is ambiguous in the literature. While we have only used fitness here to mean number of offspring per individual, empirical papers—particularly microbial ones—often use fitness to mean growth rate because that is easier and more intuitive to model. For clarity, assuming exponential growth, the number of cells at time t is:

$$n(t) = n(0)e^{\gamma t} \tag{12}$$

where γ is the growth rate and $\frac{n(t)}{n(0)}$ is the mean number of offspring per individual. Note that the growth rate is constant in this model, but the other definition of fitness increases exponentially with time.

There are advantages to both definitions and either one is acceptable as long as the author is clear as to which definition is used. Unfortunately, this is rarely the case and authors have even confused the definitions. For example, Diggle et al. (2007) verbally define the relative fitness as "the estimated growth rate of cheats relative to that of cooperators". This is fine, however the relative growth rate of defectors is given by:

$$\frac{\ln(\frac{n_D(t)}{n_D(0)})}{\ln(\frac{n_C(t)}{n_C(0)})} = \frac{\ln(e^{\gamma_D t})}{\ln(e^{\gamma_C t})} = \frac{\gamma_D}{\gamma_C} \tag{13}$$

and the equation they use for relative fitness is:

$$\frac{\frac{n_D(t)}{n_D(0)}}{\frac{n_C(t)}{n_C(0)}} = \frac{e^{\gamma_D t}}{e^{\gamma_C t}} = e^{(\gamma_D - \gamma_C)t} \tag{14}$$

which is the relative total growth of defectors and not the relative growth rate. The ambiguity is further exemplified by their explanation of their relative fitness term (w): "$w = 2$ would correspond to the mutants growing twice as fast as the wild-type cooperator." This confusion is very unfortunate because any reader that skips the math in the online supplemental methods would grossly overestimate the defector's fitness advantage, a central point of the paper. Note that this mix up was probably just a semantic issue, rather than a conceptual one.

It should be noted that while growth rate is often more intuitive to model for microbes, Hamilton's rule uses the other definition of fitness so odd transformations need to be done to use Hamilton's rule with more natural mechanistic models.

We should also mention that the relative fitness, no matter how it is defined, is much less important than the absolute fitness (Figure 2). While the relative fitness can be nice because it is only one number rather than two and because a relative fitness of 1 is fundamental, too much information is lost for it to be useful in most cases. For example, even if the relative fitness profile is the same for two populations (Figure 2A), the potential for cooperation could be drastically different (Figure 2B versus C). Also, two populations could have the same potential for cooperation, but significantly different relative fitness measurements (Figure 2D, E, and F). In addition to it being uninformative, presenting only relative fitness masks much of the non-linearity in the system. Unfortunately, the relative fitness seems to be the default value to present in the literature, making results difficult to interpret.

4 Assuming weak selection gives a different result

In kin selection models, weak selection refers to mutants varying from the wild type phenotype by an infinitesimal amount. This can be manifested by mutants producing slightly more public good or cooperating slightly more often than wild type. While weak selection is usually appropriate for animals, microbes are often under strong selection pressures and mutants can vary significantly from the wild type. It is therefore important to understand what changes when we assume weak selection.

To illustrate how assuming weak selection can give quantitatively different results, let us analyze an interaction with and without weak selection. In this interaction, we will assume that whenever a player cooperates, it pays a cost c to give a benefit b to its partner while defectors contribute nothing. Also, whenever two cooperators interact, there is an additional synergy term, d, that both cooperators receive (Figure 3). Note that any two player game with two strategies can be rephrased in this way and that the additive form of prisoner's dilemma has $d = 0$.

If the players play discrete strategies (i.e. non-weak selection), then the equilibrium fraction of cooperators is:

$$p^* = \frac{c - r(b + d)}{(1 - r)d} \tag{15}$$

which is found by setting the cooperator and defector payout equal (see Grafen (1979) for details). If, however, we assume that every individual plays a mixed strategy, p, by cooperating a fraction p of the time and defecting $1 - p$ of the time, and that any invading mutant can only mutate to $p \pm \delta$ for $\delta \to 0$ (the limit of weak selection), then we get a completely different result. Now, the payout matrix can be reduced to Figure 3C and the equilibrium level of cooperation is:

$$p^* = \frac{c - rb}{(1 + r)d} \tag{16}$$

which is clearly not the same as (15) (see Queller (1984) for derivation). Additionally, if we allow players to mutate to any mixed strategy, then spatial chaos and coexistence between multiple strategies can occur, a result that is impossible to predict using Hamilton's rule (Wenseleers, 2006).

Given that weak selection gives a different answer, why is it used? First, relatedness can often only be calculated in systems without selection, by using neutral genetic markers in empirical studies and pair-approximations in theoretical treatments. Second, the assumption of low mutation rate is used because

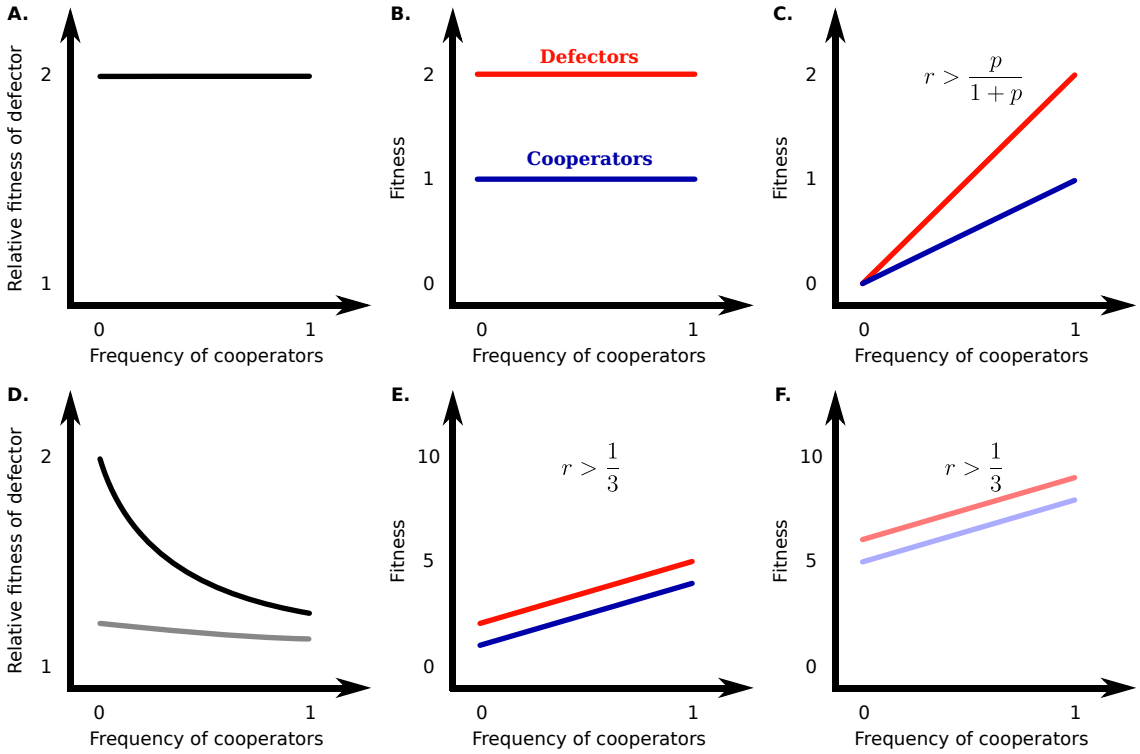

Figure 2: The relative fitness is much less informative than absolute fitness. Unfortunately, the relative fitness is what is often presented in the literature, while the absolute fitness is the quantity required for calculations and true understanding of the system. For example, even if the relative fitness of a defector is the same between two populations for all cooperator frequencies (**A**), the absolute fitnesses can be different (**B**, **C**), which means the necessary amount of population structure, or relatedness, to favor cooperation is different for the two cases (cooperation can never evolve in (**B**) but is favored if $r > \frac{p}{1+p}$ in (**C**), where p is the proportion of cooperators). Also, even if the relative fitness is different in two systems (**D**, dark and light lines), the required population structure could be the same (**E**, **F**), $r > \frac{1}{3}$. Also note that error is more complicated to measure for transformed data, which adds ambiguity to error bars on relative fitness measurements.

coexistence of strategies can not be handled by Hamilton's rule. Lastly, δ weak selection is necessary to ensure "equal gains from switching," namely that the change in fitness from switching strategies is the same independent of the opponent's strategy (Figure 3B and C). In general, the four possible payouts cannot be fit by a plane, but when only infinitesimal deviations from the mean are allowed, then the deviation from additivity scales with δ and goes to zero as $\delta \to 0$ (Wild and Traulsen, 2007). This same idea allows the linear regression coefficients in Hamilton's rule to be replaced by partial derivatives, turning strategy selection into a simple maximization problem by removing all frequency dependence (Frank, 1998). Unfortunately, as aesthetically pleasing and convenient as weak selection is, it is often unreasonable for microbes and should only be used in microbial models with caution because it gives different results.

5 Additional topics

5.1 b and c will be non-linear

Even if the terms describing a cooperative interaction are linear, the measured b and c will almost always be non-linear functions of time and the number of cooperators. By linear we mean that doubling the amount of invertase produced will double the total cost of production, c, and double the growth advantage gained from receiving this cooperative action. Because we assume everything is linear and we are only dealing with

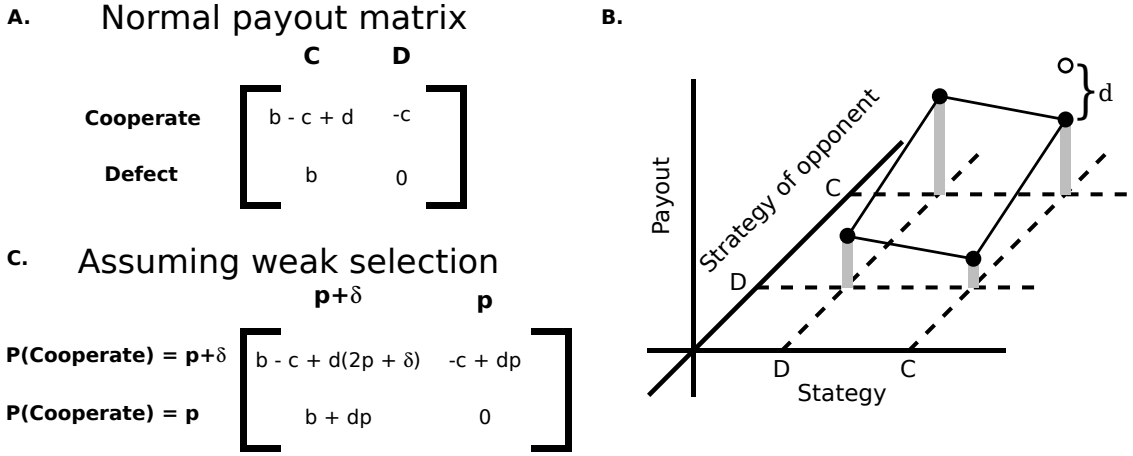

Figure 3: Assuming weak selection gives different results by making the interaction additive. (**A**) The payout given to the row player when it plays against the column player's strategy is given by the payout matrix, a standard visualization tool in game theory. The payouts for any interaction between two players with two strategies can be described using three parameters: a benefit b, cost c, and synergy term d. Here, d represents the deviation from additivity and setting d to zero would give the additive version of prisoner's dilemma or, equivalently, tragedy of the commons. (**B**) Four points cannot, in general, be fit by a plane, which is the "equal gains from switching" condition necessary for many inclusive fitness arguments to work, namely that the gain in fitness by switching one's strategy is the same independent of the opponent's strategy. (**C**) However, if δ weak selection is assumed, then the two strategies are arbitrarily close and the payouts can be well approximated by a plane because the non-additivity scales with $\delta \to 0$. The payout matrix now becomes additive because the sum of the top left and bottom right payouts equals the sum of the bottom left and top right. Note that several simplifications were made in (**C**), including subtracting the bottom right element and then dividing by δ, none of which affect the direction of selection (see Queller, 1984, for the full payout matrix).

predefined costs and benefits, the growth rate of a cooperator and defector (γ_C and γ_D) are:

$$\gamma_C = \gamma_0 + Bp_C - C \tag{17}$$
$$\gamma_D = \gamma_0 + Bp_C \tag{18}$$

where γ_0 is the base growth rate and p_C is the proportion or number of cooperators that interact with each. Notice that we use B and C here to represent benefit and costs because they will not be same as b and c used in Hamilton's rule, in general. Now, the growth of a cooperator and defector as a function of time is:

$$n_C(t) = e^{\gamma_C t} = e^{(\gamma_0 + Bp_C - C)t} \tag{19}$$
$$n_D(t) = e^{\gamma_D t} = e^{(\gamma_0 + Bp_C)t} \tag{20}$$

Simplifying and taking the Taylor series of the exponential gives:

$$n_C(t) = e^{\gamma_0 t}\left[e^{(Bp_C - C)t}\right] = e^{\gamma_0 t}\left[1 + (Bp_c - C)t + \mathrm{O}((Bp_c t - Ct)^2)\right] \tag{21}$$
$$n_D(t) = e^{\gamma_0 t}\left[e^{Bp_C t}\right] = e^{\gamma_0 t}\left[1 + Bp_c t + \mathrm{O}((Bp_c t)^2)\right] \tag{22}$$

Thus, $c = n_D - n_C \approx Ct$ and $b = \frac{\partial n_D}{\partial p_C} \approx Bt$ if C, B, and $t \ll 1$. This is the limit of weak selection, which will not hold for most realistic microbial interactions. We also assumed that the underlying interaction was linear, which is also almost never true. For example, we assumed that doubling the amount of invertase would double the amount of glucose available which in turn doubled the growth rate advantage, an immediately invalid assumption; the growth rate as a function of available glucose is non-linear (Gore et al., 2009). Linear dynamics are a very special case and should be seen as an exception rather than a rule in microbial cooperation.

5.2 Inclusive and Neighbor-modulated fitness

A major distinction never made in the text is the difference between inclusive fitness and neighbor-modulated fitness, two conceptually different, but mathematically equivalent ways to analyze Hamilton's rule. Inclusive

fitness refers to the sum of all the fitness effects a focal individual has on members of the population, weighted by the relatedness between the individuals. The gain in inclusive fitness from a cooperative act is $r \cdot b + 1 \cdot (-c) = rb - c$ because the relatedness to yourself is always 1. Thus, according to inclusive fitness theory, a trait is favored by natural selection only when it results in a gain in inclusive fitness. On the other hand, neighbor-modulated fitness focuses on all the effects on a focal individual due to the assortment in the environment. First, if the focal individual is a cooperator, it pays a cost c and every time it interacts, the chance for the other individual to also be a cooperator is r more than when a defector interacts (because of assortment). The fitness of the focal cooperator relative to a defector is then $rb - c$ versus 0 for a defector; again, cooperators spread when $rb - c > 0$. Although inclusive fitness is the one most often verbally presented in the literature, we are personally more comfortable with neighbor-modulated fitness because it is similar to game theory approaches and the fundamental idea of assortment. In fact, neighbor-modulated fitness (also referred to as direct fitness) has emerged to be the favored technique by kin selection theorists (Taylor et al., 2007).

5.3 Whole-group vs. Other-only traits

In the theoretical literature, particularly those pertaining to kin selection, a strong distinction is made between "whole-group traits" and "other-only traits." A whole-group trait is any cooperative act that the whole group benefits from, including the actor, while other-only traits are those where only other group members benefit from. Perhaps the main reason for this distinction is that other-only traits cannot evolve when groups are formed randomly, but whole-group traits can (West et al., 2006). However, both can evolve when randomly formed groups are maintained for more than a generation, when populations are seeded by clumps of more than one cell from a cell line rather than single cells, or when effects are non-linear (Fletcher and Zwick, 2004). Because all of these are likely to happen with microbes, we share the doubt of recent authors on the empirical usefulness of this distinction (Fletcher and Doebeli, 2009).

5.4 Fixation probability

We have unfortunately neglected many of the techniques used in evolutionary game theory; in particular, we have avoided the idea of fixation probability, the probability for a single mutant to take over the population. Note that because of symmetry, the fixation probability for a neutral mutant is $\frac{1}{N}$, where N is the population size (for simple population structures, otherwise see Lieberman et al., 2005). In the limit where mutations are very rare such that populations get fixed for a mutation long before a new mutation arises, cooperators are favored over defectors when they have a higher fixation probability. Thus, comparing fixation probabilities becomes equivalent to comparing which strategy will be more prevalent on average. It should be mentioned that in general, there is no relationship between the fixation probability of a cooperator and that of a defector; one could be higher than neutral, $\frac{1}{N}$, while the other is lower, or both could be higher or both lower than neutral. In contrast, in the δ weak selection limit, the probability for a $p + \delta$ mutant to fix in a population full of wild type p is $\frac{1}{N} + \xi$, where ξ is a small positive or negative number, and the fixation probability for a p mutant in a $p + \delta$ population is $\frac{1}{N} - \xi$, with the same ξ. This is a result of the "equal gains from switching" condition imposed by δ weak selection. The same condition holds in w weak selection when the underlying interaction is equal gains from switching, but not otherwise (Wild and Traulsen, 2007). For more information on evolutionary game theory see Hofbauer and Sigmund (2007); Nowak (2006); Hauert (2008).

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