Supporting Online Material: Materials and Methods

Derivation of the generalized Hamilton's rule. Our analysis is a neighbor-modulated formulation of kin selection, which partitions fitness into the effect of an individual's own genotype and the effect of social neighbors (S*1, 2*). We consider the situation in which there are two genotypes: a cooperator and a noncooperator. The social neighborhoods of these genotypes contain varying frequencies of cooperators. Social selection changes the abundance of the two strains, but mutation, recombination, and horizontal gene transfer are assumed rare enough to not significantly affect genotype frequencies.

Let the absolute fitness of a genotype be $w = n'/n$, where n and n' are the total number of individuals of that genotype before and after selection. Let the g be the genotypic value of individuals such that cooperators have $g = 1$ and noncooperators $g = 0$. Let G be an individual's social environment—the frequency of cooperators among other members of the social group. Because we are interested in social evolution, the fitness of individuals is affected by both their own genotype and that of their neighbors: $w = w(g,G)$. Any smooth fitness function can be expanded in a Taylor series around $(g = 0, G = 0)$ as

$$w = \sum_{j=0}^{\infty} b_j G^j + \sum_{k=0}^{\infty} d_k g G^k .$$

We let baseline fitness be $b_0 = a$ and the cost of cooperation when all neighbors are noncooperators be $d_0 = -c$. The covariance between fitness and genotype $\mathrm{Cov}(w, g)$ (S*1*) is then

$$\mathrm{Cov}(a,g) + \sum_{j=1}^{\infty} b_j \mathrm{Cov}(G^j,g) - c\,\mathrm{Cov}(g,g) + \sum_{k=1}^{\infty} d_k \mathrm{Cov}(gG^k,g).$$

Because a is a constant, $\mathrm{Cov}(a, g) = 0$. $\mathrm{Cov}(g, g) = \mathrm{Var}(g)$. Dividing by $\mathrm{Var}(g)$,

$$\frac{\mathrm{Cov}(w,g)}{\mathrm{Var}(g)} = \beta_{wg} = \sum_{j=1}^{\infty} b_j \beta_{G^j g} - c + \sum_{k=1}^{\infty} d_k \beta_{(gG^k)g} .$$

We let $r_j = \beta_{G^j g} = \mathrm{E}(G^j_{\mathrm{coop}}) - \mathrm{E}(G^j_{\mathrm{non}}) = m_{\mathrm{j}}^{\mathrm{coop}} - m_j^{\mathrm{non}}$, where $m_j^{(i)}$ is the jth moment of the distribution of G around $G = 0$ for genotype i. The regression definition of kin selection relatedness $r = \beta_{Gg}$ (S*1*) is equivalent to the first-order term r_1. Higher order terms $r_j = \beta_{G^j g}$ can

be thought of as higher-order relatednesses. We can write the vector of moments as $\mathbf{m}_i = \{m_1^{(i)}, m_2^{(i)}, \ldots\}$ and the vector of relatednesses as $\mathbf{r} = \{r_1, r_2, \ldots\} = \mathbf{m}_{\text{coop}} - \mathbf{m}_{\text{non}}$. If we let $\mathbf{b} = \{b_1, b_2, \ldots\}$ then $\sum b_j \beta_{G^j g} = \mathbf{r} \bullet \mathbf{b}$. Similarly, we can write $\beta_{(gG^k)g} = \text{E}(g_{\text{coop}} G^k_{\text{coop}}) - \text{E}(g_{\text{non}} G^k_{\text{non}}) = 1 \cdot \text{E}(G^k_{\text{coop}}) - 0 \cdot \text{E}(G^k_{\text{non}}) = m_k^{(\text{coop})}$. If we let $\mathbf{d} = \{d_1, d_2, \ldots\}$ and $\mathbf{m}_{\text{coop}} = \mathbf{m}$ then $\sum d_k \beta_{(gG^k)g} = \mathbf{m} \bullet \mathbf{d}$.

Selection favors cooperation when $\beta_{wg} > 0$. That is, when

$$\mathbf{r} \bullet \mathbf{b} - c + \mathbf{m} \bullet \mathbf{d} > 0. \qquad \text{(S1)}$$

In the special case where all fitness effects are completely additive (Fig. 1A), $w = a + b_1 G - cg$. Then $\mathbf{b} = \{b_1, 0, 0, \ldots\}$ and $\mathbf{d} = \{0, 0, \ldots\}$. Substituting into equation (S1) recovers the standard expression for Hamilton's rule: $r_1 b_1 - \text{c} > 0$. Mean fitness is $\overline{w} = \overline{g}\ \text{E}(w_{\text{coop}}) + (1 - \overline{g})\ \text{E}(w_{\text{non}}) = \overline{g}\,[a - c + \mathbf{m} \bullet (\mathbf{b} + \mathbf{d})] + (1 - \overline{g})[a + \mathbf{b} \bullet \mathbf{m}_{\text{non}}] = a + \mathbf{m}_{\text{non}} \bullet \mathbf{b} + \overline{g}\,\beta_{wg}$.

Bacterial strains. *Myxococcus xanthus* strains were obtained from G. J. Velicer (Indiana University). GJV1 is a descendant of the standard laboratory strain DK1622 (S*3*). GJV10 (S*4*) is a derivative of GJV1 with an integrated pDW79 plasmid that confers resistance to kanamycin. In this paper we refer to GJV10 as the cooperator strain. GJV206.3 is a laboratory-evolved cheater strain that is resistant to rifampicin (S*5*). Strains were stored at –80 C in 20% (v/v) glycerol.

Sporulation assay. Cells were grown in 8 ml CTT growth media (S*3*) at 32 ºC while shaking at 300 rpm. Log-phase cells were centrifuged 15 min at 4500 ×g and resuspended in TPM starvation media to a density of 5×10^9 cells/ml. Resuspended cells were mixed at cooperator frequencies of 0%, 1%, 10%, 50%, 90%, 99%, or 100% of total cells. 100 μl (5×10^8 cells/ml) of each cell suspension was plated onto 1.5% TPM agar. Cells developed for 5 days at 32 ºC and 90% rh. Fruiting bodies were harvested with a sterile scalpel into 1 ml dH_20, heated 2 hr at 50 ºC to kill any remaining vegetative cells, and then sonicated to disperse spores. Spores were serially diluted in dH_2O and plated in 0.5% CTT agar. Densities of GJV10 spores were measured from colony counts on plates containing 40 μg/ml kanamycin (Sigma, St. Louis). Densities of GVB206.3 spores were measured from colony counts on plates containing 5 μg/ml rifampicin (Sigma, St. Louis). Replicate experimental blocks were conducted on separate days with cells grown independently from the same frozen stock. For each strain, absolute fitness during development is equivalent to sporulation efficiency: the number of cells surviving as

spores divided by the number of cells plated. The inclusive fitness effect was calculated as mean cooperator fitness minus mean cheater fitness.

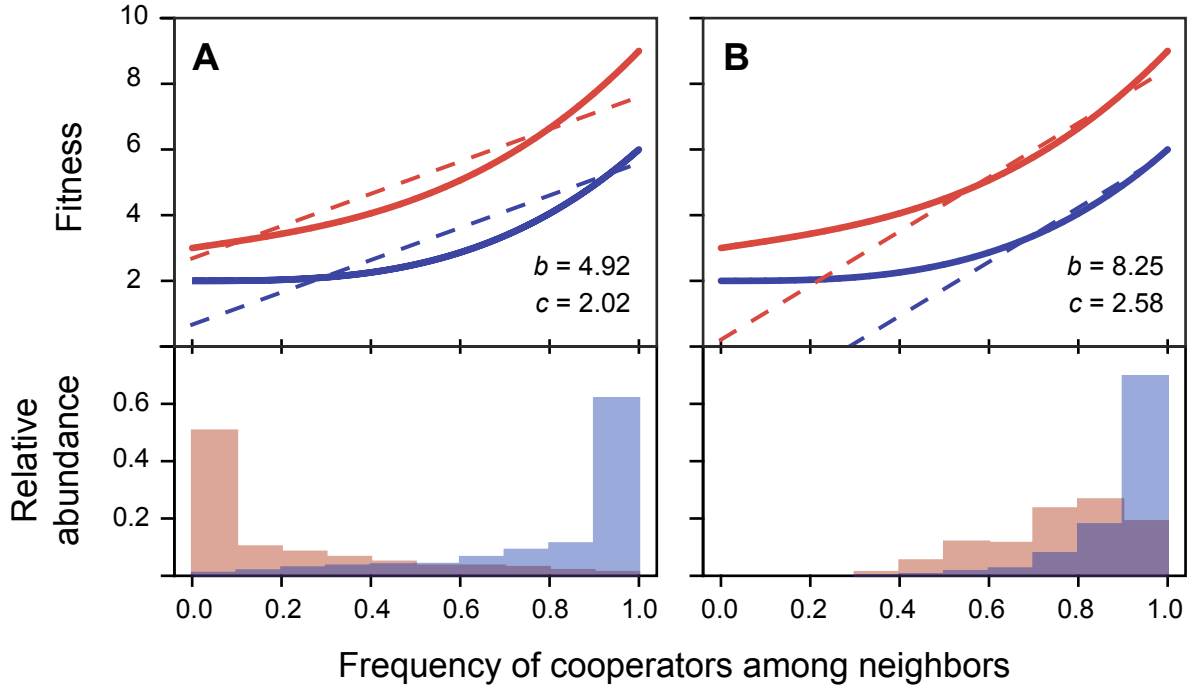
Statistics and calculations. All statistics and calculations were performed using R 2.8.1 (R Development Core Team, Vienna, Austria, http://www.R-project.org) unless otherwise indicated. The equation for developmental fitness was determined by ANCOVA (`lm` procedure) on $\log_{10}$-transformed fitness data. The best-fit statistical model included significant terms for intercept, slope (G), genotype effect on intercept (g), slope by genotype interaction ($g \times G$), and a quadratic term (G^2). $w(g, G)$ was obtained by transforming the fitted regression equation to a linear fitness scale.

Values of a, $\mathbf{b}$, c, and $\mathbf{d}$ were determined from the coefficients of the Taylor series of $w(g, G)$ up to order 30, obtained using the `Series` command in Mathematica 7.0 (Wolfram Research, Champaign, IL). We emphasize that we did not fit a 30-order polynomial to our data; we simply represented its five-parameter statistical model in terms of its Taylor series. The different components of $\mathbf{b}$ and $\mathbf{d}$ are not independent of each other. $\mathbf{m}$, $\mathbf{m}_{\text{non}}$, and $\mathbf{r}$ were calculated from the moments of the experimental distribution. If G is the initial frequency of cooperators among developing cells within a group, then moment k of genotype i was calculated as $\mathrm{E}(G^k)$, where the expectation is taken over all cells of genotype i. The inclusive fitness effect β_{wg} was calculated as in equation (S1). Error estimates were determined by bootstrap. Coefficients of the full statistical model were determined for 1000 instances of resampled data. For each of these equations, a, $\mathbf{b}$, c, $\mathbf{d}$, and β_{wg} were calculated as described above.

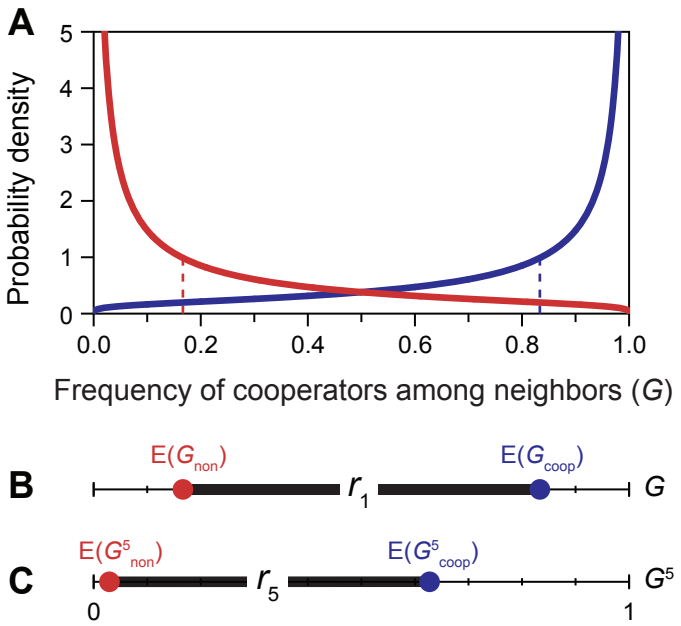
Lacking empirical data on the distribution of genotypes among naturally occuring *M. xanthus* fruiting bodies, we assumed for convention's sake an island model of population structure (S*6*) in which genotypes follow a beta distribution among groups (Fig. S2, for example). Beta distributions of within-group cooperator frequency were implemented using the `dbeta` command in R with parameters $\alpha = 2Nm\,\overline{g}$ and $\beta = 2Nm(1-\overline{g})$, where $\overline{g}$ is the global cooperator frequency and $2Nm$ is a distribution parameter. These were then normalized to obtain the distribution of G for each genotype separately: G `dbeta`$(G)/\overline{g}$ for cooperators and $(1-G)$ `dbeta`$(G)/(1-\overline{g})$ for noncooperators. Moments $m_k^{(i)}$ were calculated by numerically integrating G^k over the probability distribution of genotype i. We varied the migration parameter $2Nm$ but

report population structure in terms of first-order relatedness for ease of comparison. For each combination of parameters we calculated the inclusive fitness effect and mean fitness as described above.

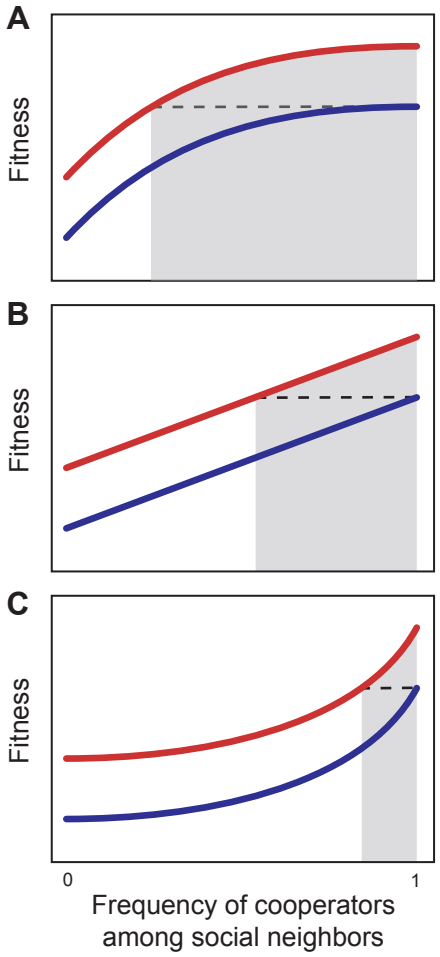
To determine the fit of Hamilton's rule to nonadditive data, we simulated island models of population structure with 500 groups of 100 individuals using the `rbeta` command in R and other parameters as described above. Each individual was assigned a fitness based on its own genotype and that of its neighbors using the fitness functions described in the text. Hamilton's rule was then fit to these distributions as a partial regression with fitness structure $w = a - cg + bG$ using the `lm` command.



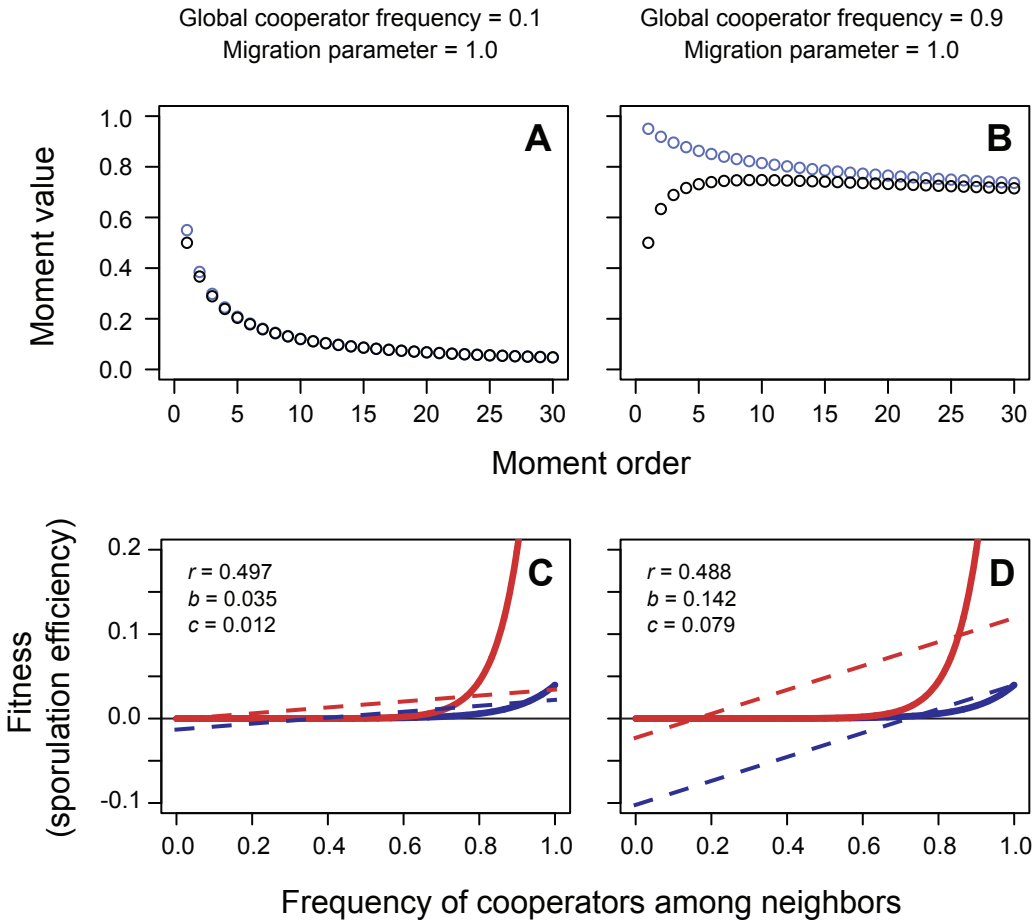
Supplementary Figure 1. Limitations of Hamilton's rule with strong nonadditivity. Solid lines in the top panels show an example nonadditive fitness function. Dashed lines show the fitness function estimated by Hamilton's rule given the distribution shown in the bottom panel. Blue: cooperators. Red: noncooperators. Hamilton's rule is effectively a linear regression fit to nonlinear data. This limits the amount of variation it can explain and in some cases leads to biologically nonsensical results like negative mean fitness at some cooperator frequencies [dashed blue line in (B)]. Hamilton's rule also confounds fitness effects with population structure: it identifies different *b* and *c* values for (A) and (B) even though they have identical fitness functions.



Supplementary Figure 2. Kin selection relatedness in asexual microbes. (A) Hypothetical distributions of cooperative genotypes among the social neighbors of cooperators (solid blue line) and noncooperators (solid red line). Dashed lines show distribution means. (B) The *r* in Hamilton's rule is r_1: the difference between the means of the distributions. (C) Higher order relatednesses are the differences between the higher-order moments of the distributions. Shown is fifth-order relatedness r_5.



Supplementary Figure 3. The functional form of nonadditive benefits determines the range of social groups in which cheaters gain a net fitness advantage over cooperators. Red line: cheater fitness. Blue line: cooperator fitness. Shaded area: cheaters have greater fitness than cooperators in all-cooperator social groups. (A) Decreasing returns from cooperation. (B) Linear returns. (C) Increasing returns. Larger shaded areas require more population structure to prevent invasion of cheaters.



Supplementary Figure 4. Identifying the causes of frequency-dependent social selection. (A, B) In the island model of population structure, kin selection relatedness (r_1) is independent of global cooperator frequency, but **r** (black) and **m** (blue) are not. Because selection in the *Myxoccoccus* example is dominated by terms of order 10-15, it is these components of population structure that create frequency-dependent selection. (C, D) Hamilton's rule misleadingly places the cause of frequency-dependent selection in its fitness terms (*b* and *c*) instead of its population structure term (*r*). Solid lines show the *Myxococcus* fitness function estimated in Fig. 2A, now plotted on a linear scale. Dashed lines show the fitness function estimated by Hamilton's rule for the population structures in the panels above. The small difference in *r* between (C) and (D) iscaused by randomness in the simulated population structures.

Supplementary References

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