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Supplementary Information for

Strategic investment explains patterns of cooperation and cheating in a microbe

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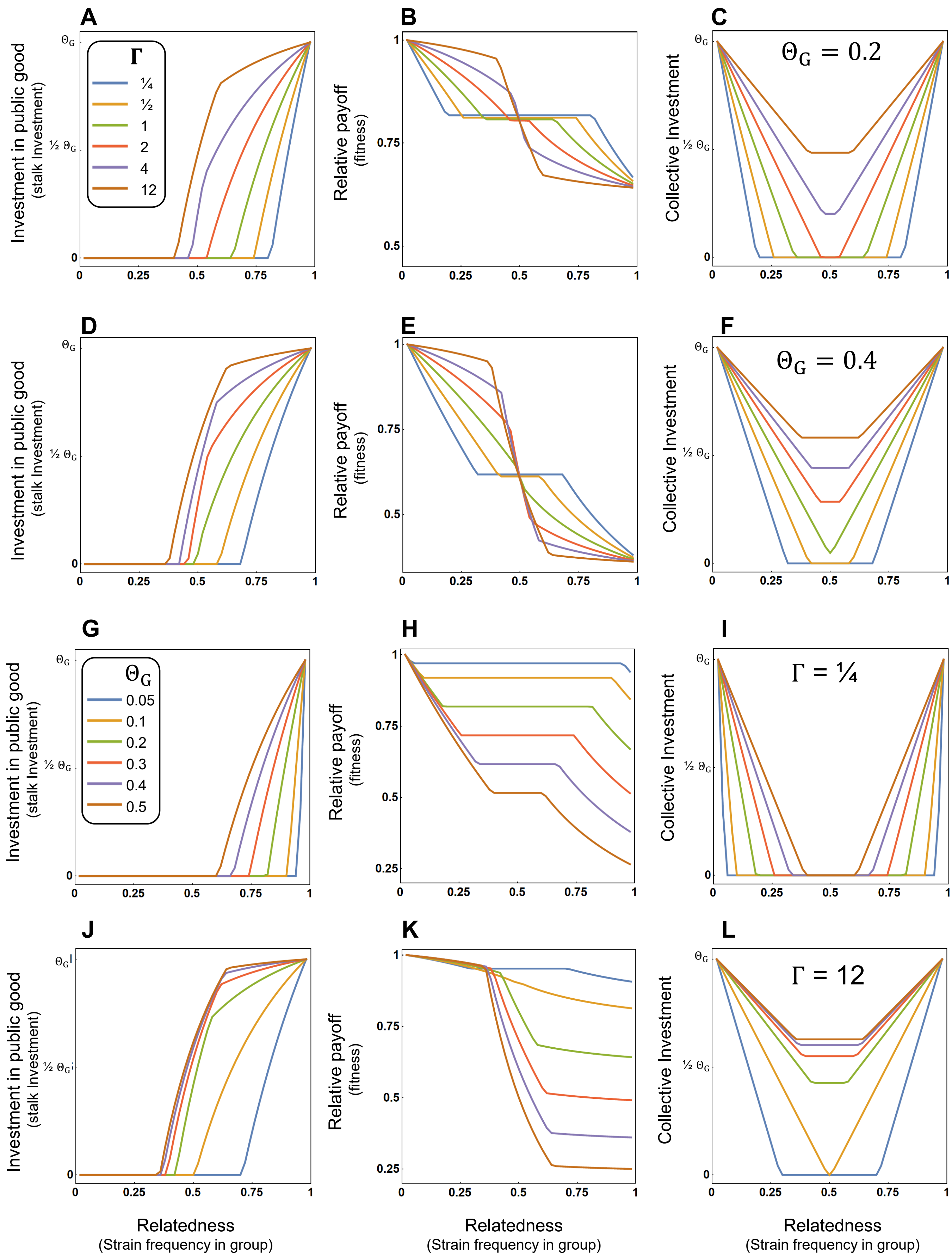
This PDF file includes:

Figs. S1 to S8

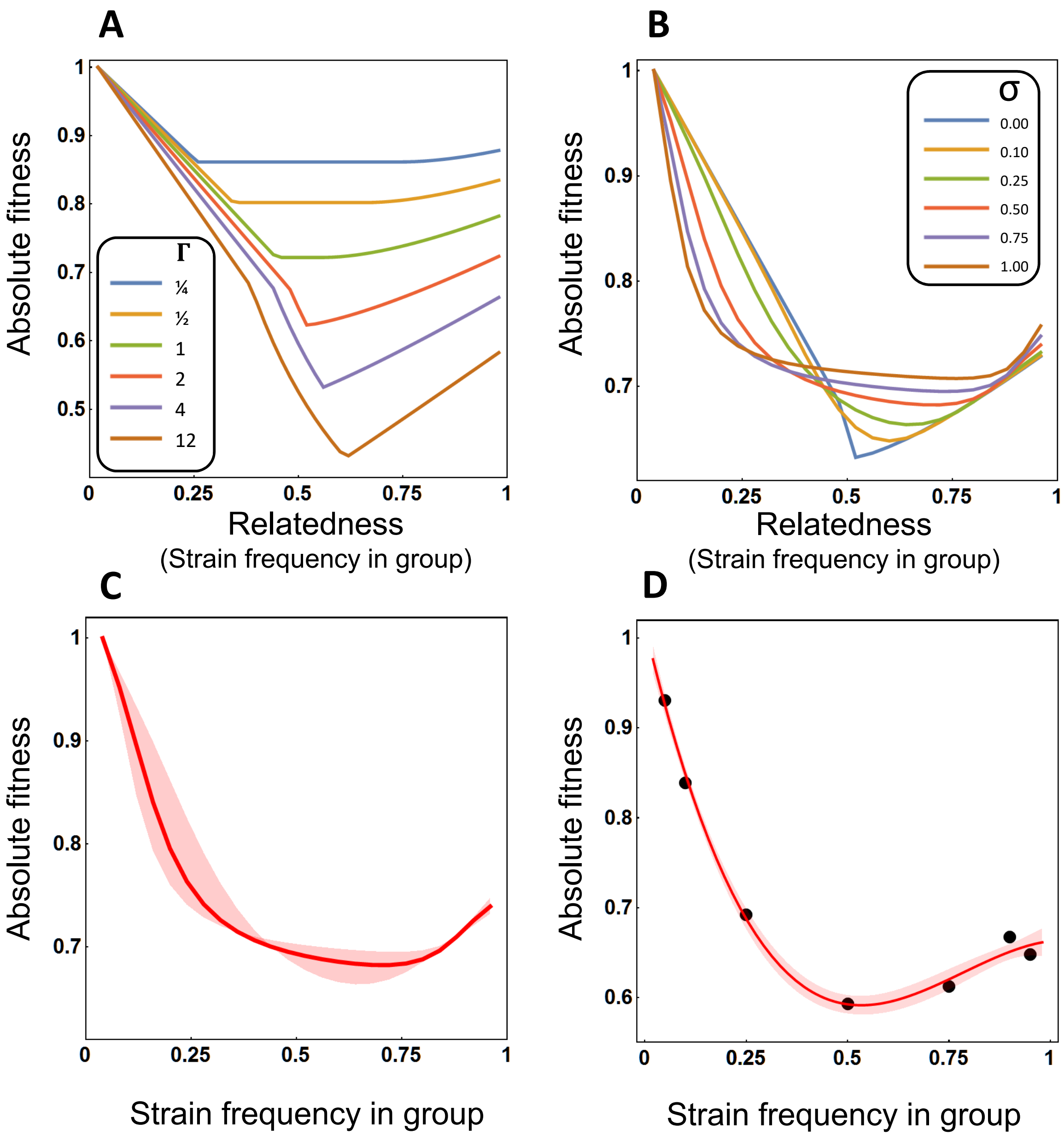
Figure S1



A
B
C
D
E
F
G
H
I
J
K
L
Γ
¼
½
1
2
4
12
$\Theta_G = 0.2$
$\Theta_G = 0.4$
Θ_G
0.05
0.1
0.2
0.3
0.4
0.5
Γ = ¼
Γ = 12
Investment in public good
(stalk Investment)
Relative payoff
(fitness)
Collective Investment
Relatedness
(Strain frequency in group)

Fig. S1. Patterns of individual investment, relative payoffs and collective investment for different optimal levels of collective investment (Θ_G) and strengths of selection (Γ). Each of the first two rows (parts A to F) show the same relationships as in Figure 2, but for two different optimal levels of collective investment ($\Theta_G = 0.2$ for A to C and $\Theta_G = 0.4$ for D to F), with lines in each figure showing the pattern expected for varying strengths of selection (Γ) (which essentially cover the entire possible range of parameter space from very weak to very strong selection on investment; the legend imbedded in the first figure of each row gives the line color of each strength of selection, with all nine panels using the same color coding). The last two rows (parts G to L) show these same relationships for two different strengths of selection ($\Gamma = ¼$ for G to I and $\Gamma = 12$ for J to L), with the lines in each figure showing the pattern for different optimal levels of collective investment. The labels in parentheses relate the figures to the patterns expected for the *D. discoideum* system. The first figure in each row (A, D, G and J) shows the pattern of individual investment into the public good (stalk investment) as a function of the player's relatedness to (frequency in) the group. The second figure in each row (B, E, H, and K) shows the pattern of relative payoff (fitness) as a function of the player's relatedness to (frequency in) the group. The last figure in each row (C, F, I, and L) shows the pattern of collective investment as a function of a focal player's relatedness to (frequency in) the group.

Figure S2



A
B
C
D
Absolute fitness
Relatedness
(Strain frequency in group)
Strain frequency in group
Γ
¼
½
1
2
4
12
σ
0.00
0.10
0.25
0.50
0.75
1.00
0
0.25
0.5
0.75
1
0.5
0.6
0.7
0.8
0.9

Fig. S2. Patterns of absolute fitness as a function of relatedness (frequency in a group). **A**) Patterns of fitness (which represents the total payoff) as a function of relatedness (frequency in a group) when individuals show the ESS pattern of investment in the public good. All patterns were calculated for the same optimal level of investment ($\Theta_G = 0.3$), with the different lines corresponding to different strengths of selection (Γ). **B**) Patterns of absolute fitness as a function relatedness (frequency) with varying degrees of error in measurement of relatedness (frequency). All patterns were calculated for the same optimal level of investment ($\Theta_G = 0.3$) and the same strength of selection ($\Gamma = 2$), with different values of error (σ). **C**) The pattern of absolute fitness calculated following the same method used to process the experimental data. Parameter values match those used in Figure 4. **D**) Experimental estimates of absolute fitness based on the patterns of stalk investment (Figure 4D) and the probability of fruiting body collapse (Figure 6A). The black points represent the estimates at the measured frequencies. The line represents the best fit line based on a cubic regression using these estimates, with the shaded region indicating one standard error on either side of the best fit line.

Figure S3

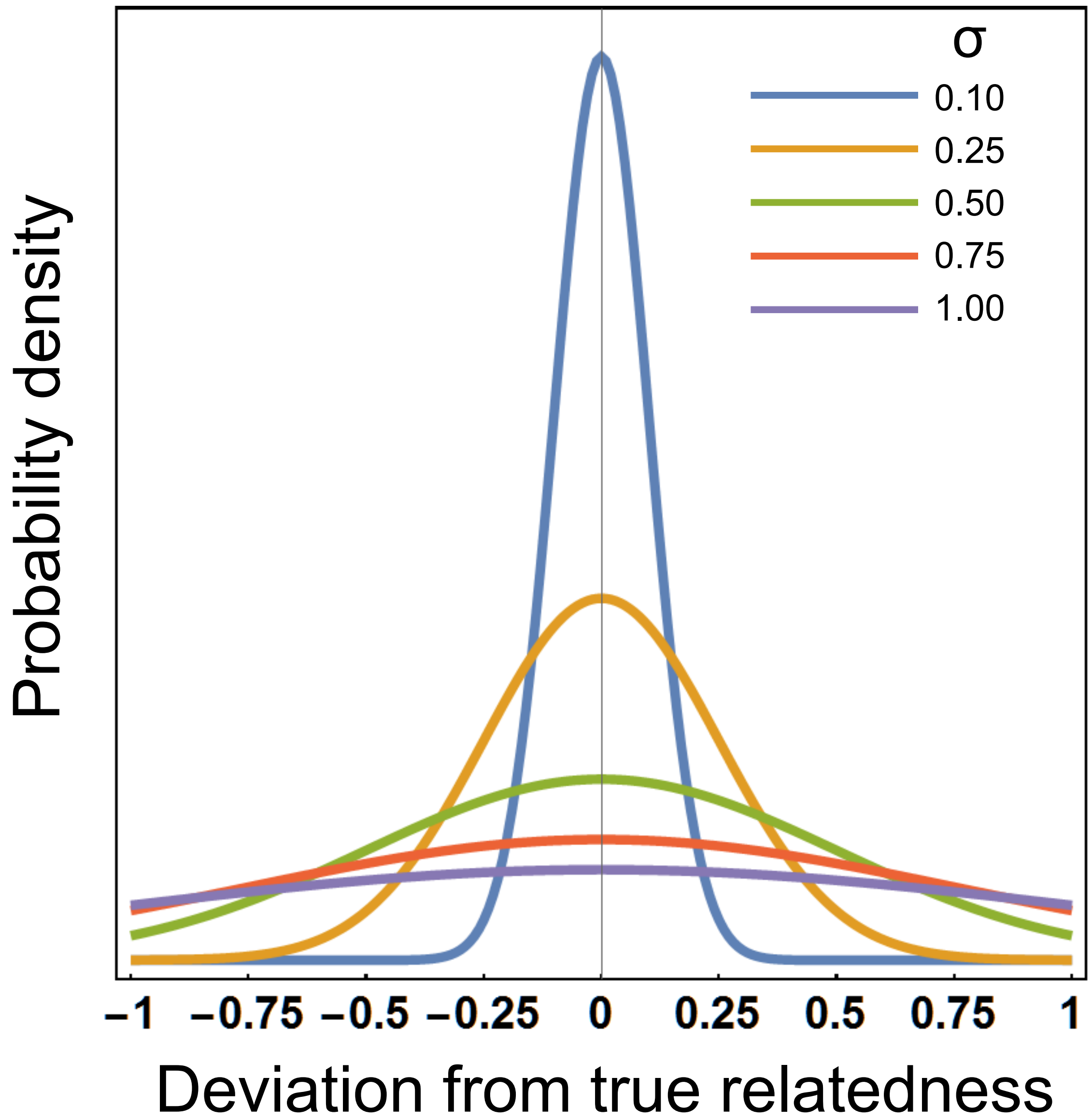
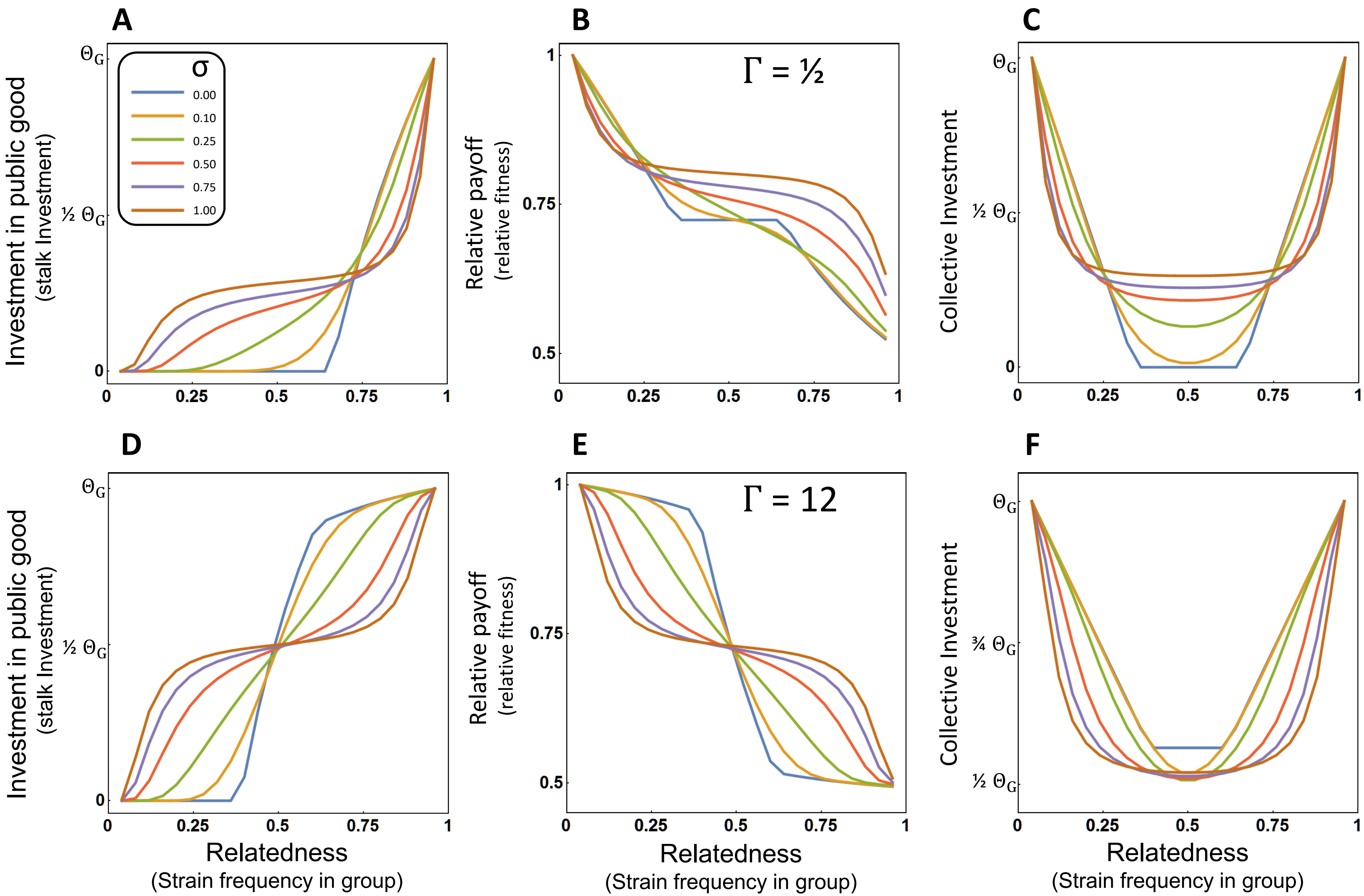

σ
0.10
0.25
0.50
0.75
1.00
Probability density
−1
−0.75
−0.5
−0.25
0
0.25
0.5
0.75
1
Deviation from true relatedness

Fig. S3. The distributions of errors in players' measurement of relatedness (frequency) that were used to generate patterns under imperfect information. Illustrated are five different probability density functions that differ in the level of error in the measurement of relatedness (frequency) (σ). The distributions are Gaussian with a mean of zero and a standard deviation given by σ. The deviations represent the difference between the relatedness (frequency) that player estimates for their group and their true relatedness. Because relatedness (frequency) is constrained to the range of zero to one, the distribution is necessarily truncated when used in calculations. For example, if a player's true relatedness is 0.25, the deviations will be truncated at −0.25 and 0.75 (where the mean of zero indicates that they have correctly measured their relatedness as 0.25).

Figure S4



A
B
C
D
E
F
σ
0.00
0.10
0.25
0.50
0.75
1.00
Γ = ½
Γ = 12
Investment in public good
(stalk Investment)
Relative payoff
(relative fitness)
Collective Investment
Θ_G
½ Θ_G
¾ Θ_G
0
0.25
0.5
0.75
1
Relatedness
(Strain frequency in group)

Fig. S4. Predicted patterns of individual investment, relative payoffs and collective investment for different strengths of selection (Γ) and levels of error in measurement of relatedness (frequency) (σ). The general structure of the figures follows that of Figure S1, except the rows show the same relationships for two different strengths of selection (Γ = ½ for A to C, and Γ = 12 for D to F), which, combined with the values illustrated in Figure 3 essentially cover the entire range of parameter space. The lines in each figure show the pattern expected for varying amounts of error in the measurement of relatedness (frequency) (σ) (see the legend imbedded in the first figure). All examples were calculated for the same optimal level of collective investment (Θ_G = 0.3) since the exact optimum has a minor effect on the patterns (see Figure 2 and S1). The labels in parentheses relate the figures to the patterns expected for the *D. discoideum* system.

Figure S5

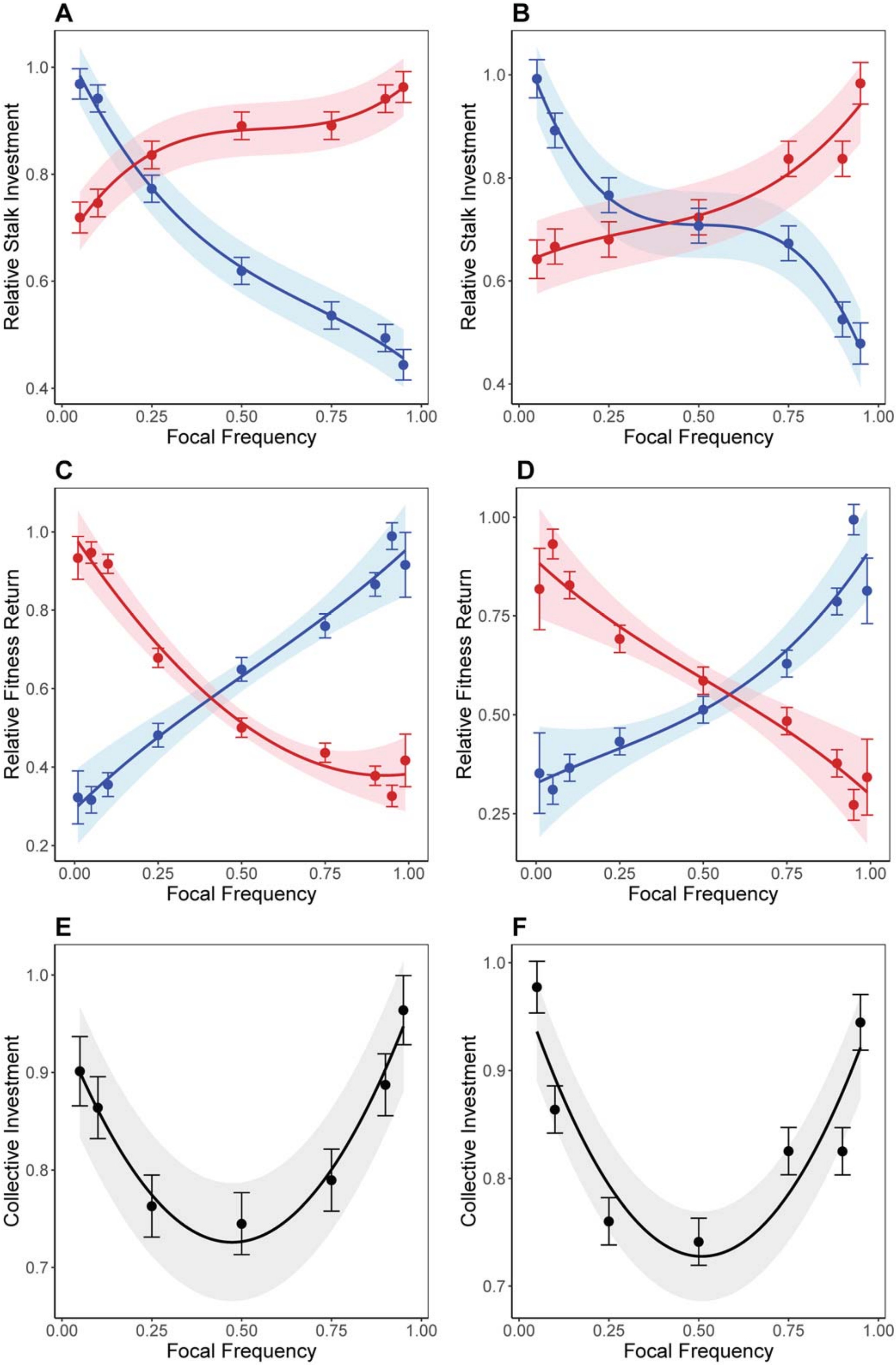

A
B
C
D
E
F
Relative Stalk Investment
Relative Fitness Return
Collective Investment
Focal Frequency
0.00
0.25
0.50
0.75
1.00
0.4
0.6
0.8
1.0
0.2
0.7
0.9
0.25
0.50
0.75
1.00

Fig. S5. Patterns of stalk investment, relative fitness, and collective investment as a function of the frequency of a strain in a chimeric aggregation for two high resolution pairs of natural strains combined in chimeras. **A)** Stalk investment ($I_{i|p_i}$) by NC105.1 (in red) and NC34.2 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC105.1 (designated as the focal strain) in the mix, **B**) Stalk investment ($I_{i|p_i}$) by NC63.2 (in red) and NC28.1 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC63.2 (designated as the focal strain) in the mix. **C)** Relative fitness (ρ_i) of NC105.1 (in red) and NC34.2 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC105.1 in the mix, **D**) Relative fitness (ρ_i) of NC63.2 (in red) and NC28.1 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC63.2 in the mix. **E)** Collective stalk investment (I_G) by chimeras composed of NC105.1 and NC34.2 as a function of the frequency of NC105.1 in the mix, **F)** Collective stalk investment (I_G) by chimeras composed of NC 63.2 and NC28.1 as a function of the frequency of NC63.2 in the mix. Each panel shows the estimated means (with their standard errors) at each frequency measured (with values estimated by the mixed model describe in the Methods, but using frequency as a categorical factor). In all cases, the y-axis values are scaled as proportions of the maximum value observed. In parts A-D the bold curve represents the best-fit estimated from the cubic regression model (here fitted to the estimated means) and the like-colored shaded regions give approximate 95%-confidence intervals around those curves. For parts E and F the curve represents the best-fit estimate from a quadratic regression model (fitted to the estimated means) and the shaded region gives the 95% confidence interval around that relationship.

Figure S6

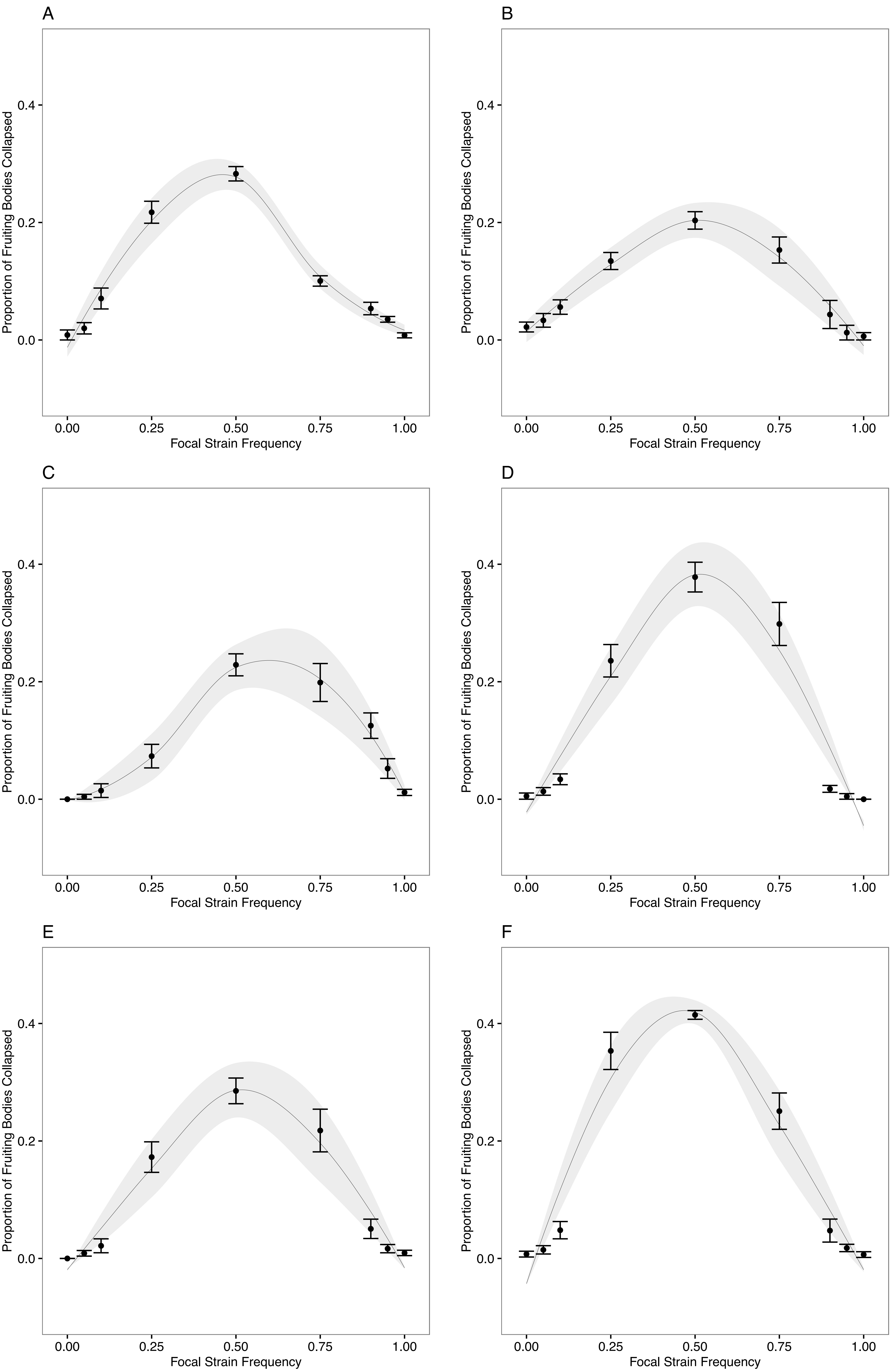

A
B
C
D
E
F
Proportion of Fruiting Bodies Collapsed
0.0
0.2
0.4
Focal Strain Frequency
0.00
0.25
0.50
0.75
1.00

Fig. S6. Spontaneous fruiting body collapse as a function of focal strain frequency for six strain pairs. Points represent the mean observations (with standard error bars) and the curve illustrates the best-fit polynomial relationship (with 95%-confidence intervals as grey-shading). The six pairs appear as: **A)** NC28.1+NC105.1, **B)** NC60.1+NC99.1, **C)** NC34.2+NC105.1, **D)** NC99.1+NC105.1, **E)** NC60.1+NC34.2 and **F)** NC60.1+NC63.2.

Figure S7

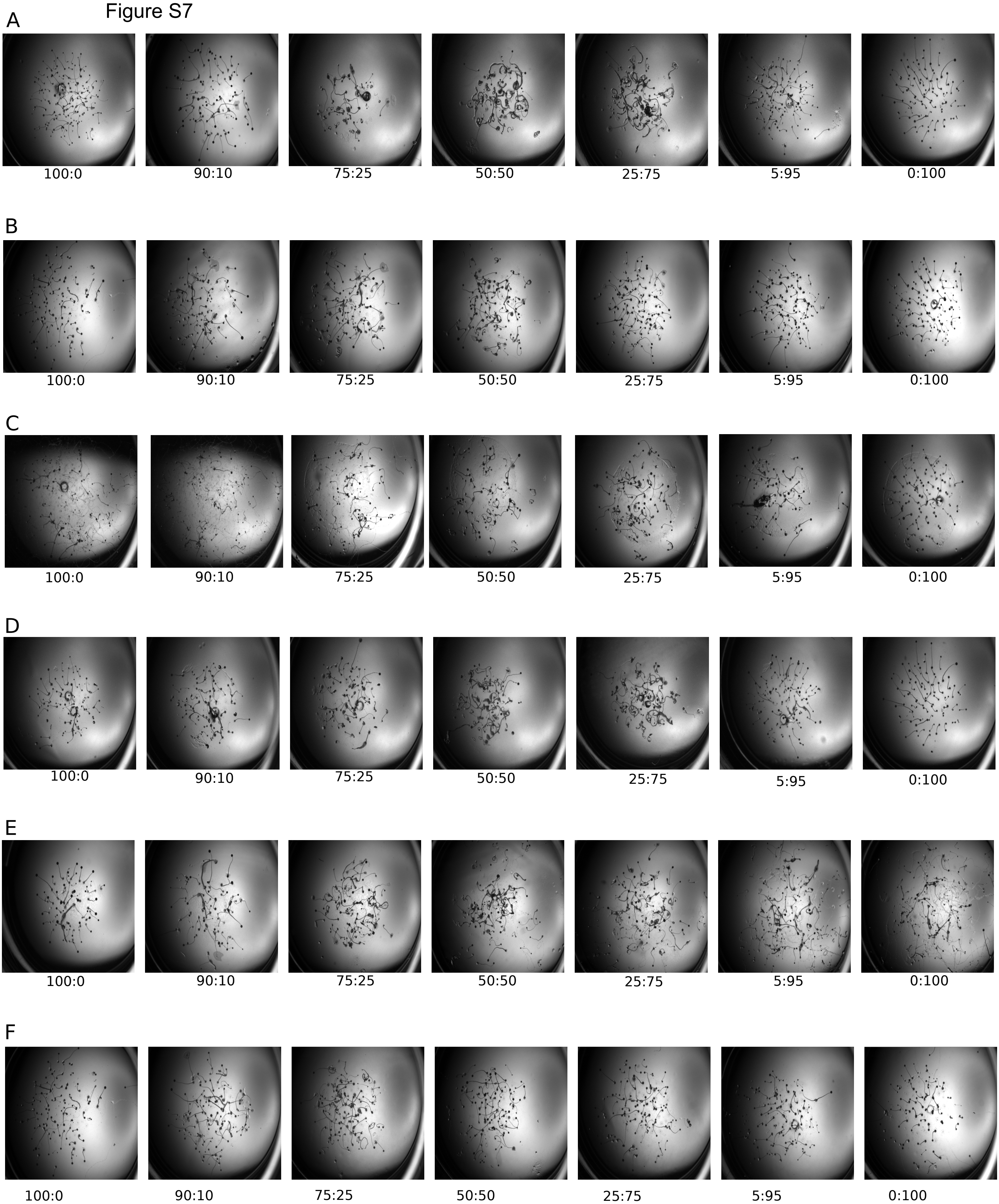
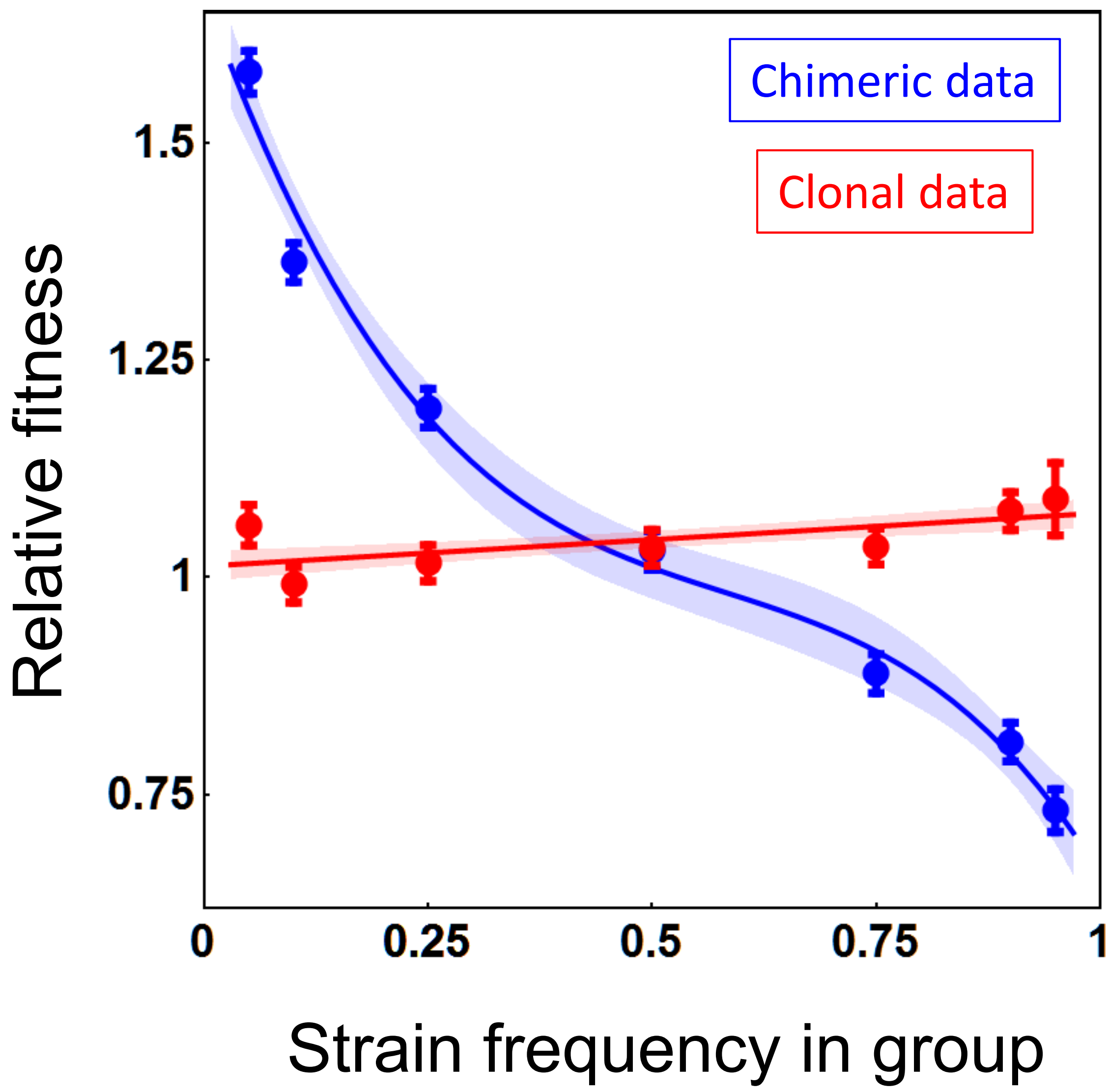

A
100:0
90:10
75:25
50:50
25:75
5:95
0:100
B
100:0
90:10
75:25
50:50
25:75
5:95
0:100
C
100:0
90:10
75:25
50:50
25:75
5:95
0:100
D
100:0
90:10
75:25
50:50
25:75
5:95
0:100
E
100:0
90:10
75:25
50:50
25:75
5:95
0:100
F
100:0
90:10
75:25
50:50
25:75
5:95
0:100

Fig. S7. Representative images of fruiting bodies for six strain pairs across a range of focal strain frequencies. **A)** NC34.2+NC105.1, **B)** NC60.1+NC34.2, **C)** NC60.1+NC63.2, **D)** NC99.1+NC105.1, **E)** NC60.1+NC99.1 and **F)** NC28.1+NC105.1).

Figure S8



Chimeric data
Clonal data
1.5
1.25
1
0.75
Relative fitness
0
0.25
0.5
0.75
1
Strain frequency in group

Fig. S8. The pattern of relative fitness as a function of frequency in a group. The parts in blue correspond to the estimated pattern for chimeric mixes. They therefore match the pattern of relative fitness shown in Figure 4E and are included here for comparison. The parts in red correspond to the pattern for clonal self-mixes, which were estimated following the same method used to calculate the chimeric pattern, with the labelled cells considered as the 'focal' strain. The points indicate the means and the bars their standard errors, both estimated from a mixed model. The lines represent the best fit relationship (which is cubic for the chimeric data and linear for the clonal data), with the shaded region indicating one standard error on either side. The slope of the best fit line for the clonal data is not significant ($F_{1, 195} = 1.65$, $p = 0.2$, but is included as an illustration of the relationship.