LETTERS

Resource competition and social conflict in experimental populations of yeast

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Understanding the conditions that promote the maintenance of cooperation is a classic problem in evolutionary biology[1–5]. The essence of this dilemma is captured by the 'tragedy of the commons'[6]: how can a group of individuals that exploit resources in a cooperative manner resist invasion by 'cheaters' who selfishly use common resources to maximize their individual reproduction at the expense of the group[7,8]? Here, we investigate this conflict through experimental competitions between isogenic cheater and cooperator strains of yeast with alternative pathways of glucose metabolism[9], and by using mathematical models of microbial biochemistry[10]. We show that both coexistence and competitive exclusion are possible outcomes of this conflict, depending on the spatial and temporal structure of the environment. Both of these outcomes are driven by trade-offs between the rate and efficiency of conversion of resources into offspring that are mediated by metabolic intermediates.

Heterotrophic microorganisms depend on metabolic pathways to convert resources, such as sugars, into energy in the form of ATP, which is required for growth and reproduction. Trade-offs between the rate and yield of substrate degradation set the stage for evolutionary conflict between individuals with alternative metabolic pathways that convert resources into energy either rapidly or efficiently[7,8]. Efficient use of common resources conforms to the classical definition of a cooperative trait: it is beneficial to the group, because more biomass is produced per unit resource, but costly to individuals, because each cell produces energy and divides at a slow rate.

Under what conditions can a group of cooperating microbial cells resist invasion by cheaters who wastefully deplete common resources to increase their growth rate? Theoretical studies have addressed this question by modelling competition between cooperative respirers that take up glucose slowly and fully respire all of the glucose they ingest, resulting in a high yield of ATP production, and selfish respiro-fermenters that take up glucose rapidly and ferment any excess glucose that cannot be respired, resulting in a high rate of ATP production[7,8,11]. In these models the outcome of competition depends on the scale of resource competition: cheaters exclude cooperators when competition is for a global pool of resources in a homogeneous environment; cooperators resist invasion by cheaters if competition occurs for local resource patches in a spatially structured environment[7,8,11].

To investigate the outcome of global competition, we competed isogenic respirer (cooperator) and respiro-fermenter (cheater) yeast strains[9,12] in glucose-limited chemostats. The cheater has a higher fitness than the cooperator in the chemostat (mean $w = 1.08$, s.e.m $= 0.022$, $t_{(1),11} = 3.43$, $P = 0.0028$) and competition results in exclusion of the cooperator. To investigate the effect of seasonality on global competition, we carried out a second set of experiments in glucose-limited batch cultures. Fitness in batch cultures is negative frequency-dependent ($F_{1,46} = 58$, $P < 0.0001$, $r^2 = 0.59$; Fig. 1a) and both strains are mutually invasible, implying that the outcome of competition is stable coexistence. Competitive fitness of the cooperator in batch cultures is also positive density-dependent ($F_{1,18} = 12.3$, $P = 0.002$; Fig. 1b), implying that the cooperator achieves a higher equilibrium frequency when population density at the start of a season is high.

To investigate mechanisms of coexistence, we developed a seasonal model of competition for a sugar. For simplicity, we model the catabolism of sugar and its intermediates as a two-reaction process[10] corresponding to glycolysis and the tricarboxylic acid (TCA) cycle. In

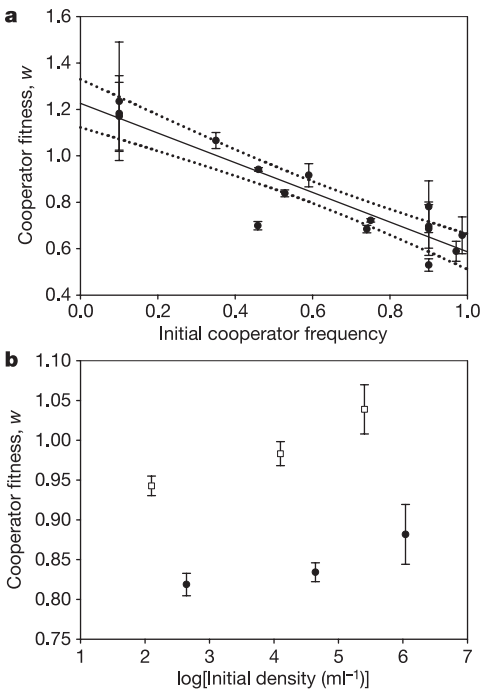

Figure 1 | Seasonal competition between cooperators and cheaters. **a**, Plotted points show cooperator fitness (±s.e.m; $n = 3$ or 4) as a function of initial frequency. The solid line shows the regression of fitness on frequency and dotted lines show the 95% confidence interval about this regression. Initial glucose concentration varied between competitions, but the slope ($F_{1,12} = 0.79$, $P = 0.39$) and intercept ($F_{1,12} = 0.4$, $P = 0.54$) of the regression are independent of initial glucose concentration. Initial population density was constant in all competitions. **b**, Plotted points show the competitive fitness of the cooperator strain (±s.e.m; $n = 4$) at an initial frequency of 0.9 (closed symbols) or 0.4 (open symbols) across a gradient of initial population density. Initial glucose concentration was the same in all competition.

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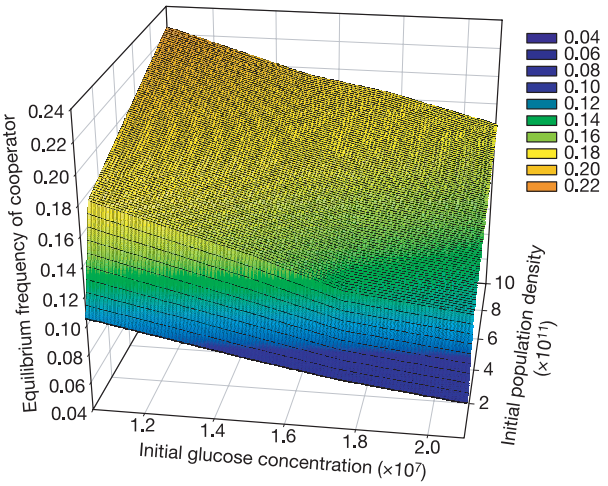

Figure 2 | Modelling competition between cheaters and cooperators. This plot shows the outcome of competition across different initial densities and glucose concentrations. The frequency of the cooperator strain at steady state is plotted against different initial densities and glucose concentrations (in nanomoles) at the beginning of each season.

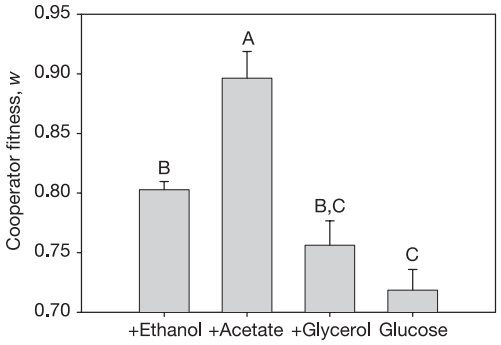

Figure 3 | Toxic metabolic intermediates create a cost of cheating. Cooperator and cheater strains were competed against each other on agar plates containing 8 g l^{-1} glucose or 8 g l^{-1} glucose supplemented with glycerol, acetate, or ethanol at a concentration of 4 g l^{-1} each. The initial population density and frequency of the cooperator were the same in all competitions. Bars show the mean competitive fitness of the cooperator strain (±s.e.m; $n = 3$). Mean fitness differs among treatments that are not connected by the same letter (A, B, C), as judged by a one-way analysis of variance, ANOVA ($F_{3,8} = 18.6$, $P = 0.0006$), followed by a Tukey test ($P < 0.05$).

the first reaction, sugar is taken from the environment and partially oxidized to form an intracellular metabolic intermediate and n_1 molecules of ATP. In the second reaction, the intracellular intermediate is either completely oxidized to form CO_2 and n_2 molecules of ATP, or it passively diffuses out of the cell as an extracellular intermediate. We assume that both catabolic reactions show saturating enzyme kinetics, that the rate of cell growth is proportional to the rate of ATP production[13] according to a proportionality constant G, and that the intracellular intermediate imposes a cost in terms of lost ATP given by a linear function g as a result of deleterious effects of metabolites such as end-product inhibition and direct toxicity[10,14] (See Supplementary Fig. 1 for the pathway schematic). We parameterized this model using data on the biochemistry of yeast, assuming that the only innate difference between strains is that cheaters have a higher maximum rate of glycolysis (Supplementary Table 1). The dynamics of this model within one season are described as follows, where S is the concentration of sugar in moles, X_{in} and X_{ex} are the concentrations of the intracellular and extracellular intermediates in moles respectively, v_1 and v_2 are the rate of glycolysis and the TCA cycle respectively, c is a transport constant, N denotes cell density, and the subscripts r and f denote cooperators and cheaters (units of measurement are presented in Supplementary Table 1):

$$
\begin{aligned}
dS/dt &= -v_{1r}(S)N_r - v_{1f}(S)N_f \\
dX_{ex}/dt &= c[X_{in,r} - X_{ex}]N_r + c[X_{in,f} - X_{ex}]N_f \\
dN_r/dt &= Gg(X_{in,r})[v_{1r}(S)n_1 + v_2(X_{in,r})n_2]N_r \\
dN_f/dt &= Gg(X_{in,f})[v_{1f}(S)n_1 + v_2(X_{in,f})n_2]N_f \\
dX_{in,r}/dt &= [-c[X_{in,r} - X_{ex}] + v_{1r}(S) - v_2(X_{in,r})]N_r \\
dX_{in,f}/dt &= [-c[X_{in,f} - X_{ex}] + v_{1f}(S) - v_2(X_{in,f})]N_f
\end{aligned}
\qquad (1)
$$

This model predicts density-dependent coexistence of both strategies in seasonal environments at frequencies similar to those observed in our competition experiments, implying that cheating carries costs and benefits (Fig. 2). The benefit of fermentation is that it gives cheaters a higher rate of ATP production for any given value of S, resulting in a high maximum growth rate[9]. The cost of this strategy is twofold. Cheaters pay a cost in terms of reduced reproductive yield (number of offspring per mole of ATP), because they are prone to accumulate high concentrations of toxic intermediates (see Supplementary Note 1 for the mathematical explanation). This cost is high when extracellular intermediates accumulate to high concentrations, because X_{ex} inhibits the diffusion of excess X_{in} out of cheater cells. Consistent with this expectation, the addition of toxic metabolites (ethanol and acetate) to batch culture competitions increases cooperator fitness, while the addition of a non-toxic metabolite (glycerol) has no significant effect on fitness (Fig. 3). The evolutionary consequence of reduced reproductive yield is that the relative growth rate (that is, the fitness) of the cheater declines as intermediates accumulate. The second cost of cheating is that it lowers the biochemical yield of energy production (moles of ATP per mole of sugar). This cost can be illustrated as follows: cooperators always gain n_2 molecules of ATP from each molecule of X_{in} they produce, while cheaters gain less than n_2 molecules of ATP from each X_{in} they produce, because some of the excess intermediates produced by cheaters are subsequently oxidized by cooperators after S has been depleted.

Seasonality promotes coexistence in this system because it allows metabolic intermediates to accumulate, reducing the biochemical and reproductive yield of energy production in cells with a high biochemical rate (moles of ATP per unit of time) of energy production. Consistent with this argument, the fitness of the cheater is lowest under conditions that promote the accumulation of high concentrations of extracellular metabolites, namely high absolute cheater density (Fig. 1). This result is of general importance to our understanding of social conflict in microbial populations because trade-offs between the rate and yield of energy production are a general feature of metabolism that are not specific to this system[7,8]. In this case, metabolic intermediates are the molecules that mediate this trade-off. Coexistence is not possible in the chemostat in this system because intermediates are constantly washed out of the chemostat, minimizing the cost of cheating (Supplementary Equation (1)), although both coexistence and competitive exclusion of cheaters are theoretically possible in chemostat models that assume much higher costs of cheating[10].

Spatial structure promotes the evolution of cooperation in game theoretical models of social conflict because it allows synergistic interactions among localized populations of cooperators to result in large payoffs that are not accessible to cheaters[5,8]. The benefit of

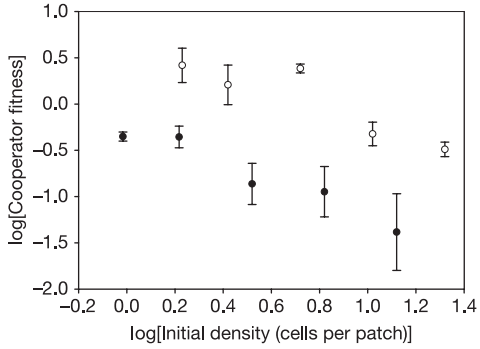

Figure 4 | Competition in a spatially heterogeneous environment. Plotted points show the mean fitness (±s.e.m; $n = 3$) of the cooperator strain at the level of the metapopulation. The initial frequency of the cooperator strain was either 0.91 (open symbols) or 0.45 (closed symbols). Because the number of generations of competition varied between patches in the metapopulations, fitness was not standardized to the number of generations of competition.

cooperation in this system is that a group of cooperators that exploit a local resource patch will produce more offspring than an equivalent population of cheaters because they have a higher reproductive yield of ATP production. In a spatially heterogeneous environment consisting of many local patches, cooperation will be favoured when (1) the frequency of the cheater is low, or (2) the number of colonists per patch is low, because many patches will be exclusively colonized by cooperators under these two scenarios.

To test this hypothesis, the cheater and cooperator strains were inoculated at random into a metapopulation made up of 96 discrete patches. After one season of growth, we pooled the populations from each patch in the metapopulation to assay the outcome of competition at the level of the metapopulation (Fig. 4). Within any given patch, competition is seasonal. In agreement with our hypothesis, the fitness of the cooperator at the level of the metapopulation is both positive frequency-dependent ($F_{1,7} = 55$, $P < 0.0001$; Fig. 4) and negative density-dependent ($F_{1,7} = 41, P < 0.0001$; Fig. 4), implying that a metapopulation of cooperators can resist invasion by a rare cheater provided that the number of cells that colonize each patch is below a critical threshold. Positive frequency-dependent selection for cooperation also implies that a rare cooperator cannot invade a population of cheaters.

Competition for common resources is one of the simplest social conflicts that can be imagined. Evolutionary game theory models based on the Prisoner's Dilemma predict that the outcome of this conflict is simple: cheating will prevail in homogeneous populations[2,7,8,11] and spatial structure will promote the evolution of cooperation[5,7,8]. This study reports two experimental findings that are of general relevance for our understanding of social conflict: (1) cooperation can persist in a well-mixed population in the absence of kin-recognition, policing or rational behaviour and (2) spatial structure can promote the maintenance of cooperation.

Social conflict is recognized as an important aspect of microbial population biology[15–18] and metabolism[17,19] even though microbes are not capable of rational thought. Both theoretical and experimental studies of these conflicts have investigated the fitness of fixed strategies, even though microbes are capable of rapid changes in gene expression in response to simple environmental cues such as substrate availability. The evolutionary optimal strategy in the conflict we describe here may be a plastic genotype that switches between selfish and efficient metabolism depending on the concentration of intermediates in the environment, instead of the 'wild-type' *Saccharomyces cerevisiae* strategy, which is to ferment any excess glucose that cannot be respired irrespective of ethanol concentration[9]. Future studies that investigate microbial social conflict at the level of individual cells will hopefully shed light on this intriguing possibility.

METHODS

Yeast strains. We used yeast strains CEN.PK2-1C and TM6*, an isogenic respirer mutant that carries a single, synthetic hexose transporter gene[9]. Both strains are haploid and MATa.

Chemostat competitions. Strains were competed over two days in chemostats supplied with glucose-limited medium (glucose 0.8 g l^{-1}, yeast nitrogen base 1.7 g l^{-1}, ammonium sulphate 5 g l^{-1}, uracil 0.002%) that were incubated at 30 °C with continous shaking (150 r.p.m.) and aeration. The dilution rate of the chemostats varied between 0.27 and 0.35 to ensure that that glucose flux was high enough to allow the cheater mutant to ferment[20].

Batch-culture competitions. Pre-inoculation cultures were mixed at various ratios to form common pools that were used to inoculate replicate competition cultures (glucose 0.25 g l^{-1}–20 g l^{-1}, yeast nitrogen base 1.7 g l^{-1}, ammonium sulphate 5 g l^{-1}), supplemented with uracil (0.002%) and agar (1.6%) where necessary. Competition cultures were incubated for two days at 30 °C; flasks were continuously shaken at a speed of 150 r.p.m.

Spatial competition. Common pools were innoculated into 96-well microplates containing 150 μl of competition medium (glucose 20 g l^{-1}, yeast nitrogen base 1.7 g l^{-1}, ammonium sulphate 5 g l^{-1}, uracil 0.002%). Microplates were incubated for 5 days at 30 °C without shaking.

Estimating fitness. The population density of the two strains at the start and end of competition was measured by plating appropriate serial dilutions on YPD agar plates. At low population density, the two strains form very large (CEN.PK2-1C) or small (TM6*) colonies. The accuracy of this technique was confirmed by replica-plating neutrally marked strains (ura-, kan^R) onto appropriate selective media. Competitive fitness was calculated as w per generation, where w is the exponent of the change in the natural logarithm of the ratio of the density of the two strains during the course of competition divided by the number of population doublings that occurred during competition, such that a value of $w = 1$ represents equal competitive ability.

Mathematical modelling. A population of N_r cooperator and N_f cheater cells was introduced into an environment containing a fixed initial concentration of resources and no metabolic intermediates. Competition occurred until all resources and intermediates were depleted from the environment. We assumed that only a proportion of the total population at the end of a season survived into the next season, in which the environment contained the same fixed resource concentration as in the previous season and no metabolic intermediates. The total cell density at the beginning of each season was kept constant and therefore the only component that changed at the beginning of each season was the cooperator/cheater ratio. The stable coexistence between cooperators and cheaters was said to be reached if the cooperator/cheater ratio remained constant between the seasons. Computer simulations were conducted using MATLAB while the model was parameterized with data on the biochemistry of the cooperator and cheater strains used in our competition experiments (Supplementary Information 1).

Received 15 December 2005; accepted 2 February 2006.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This project was funded by grants from NERC to the Center for Population Biology. I. Gudelj was supported by a NERC-EMS Fellowship. The authors would like to thank C. Godfray for comments and C. Brandon for technical assistance.

Author Contributions R.C.M. carried out experimental work and wrote the manuscript. I.G. carried out mathematical modelling.

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