LETTERS

Snowdrift game dynamics and facultative cheating in yeast

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The origin of cooperation is a central challenge to our understanding of evolution[1–3]. The fact that microbial interactions can be manipulated in ways that animal interactions cannot has led to a growing interest in microbial models of cooperation[4–10] and competition[11,12]. For the budding yeast *Saccharomyces cerevisiae* to grow on sucrose, the disaccharide must first be hydrolysed by the enzyme invertase[13,14]. This hydrolysis reaction is performed outside the cytoplasm in the periplasmic space between the plasma membrane and the cell wall. Here we demonstrate that the vast majority (~99 per cent) of the monosaccharides created by sucrose hydrolysis diffuse away before they can be imported into the cell, serving to make invertase production and secretion a cooperative behaviour[15,16]. A mutant cheater strain that does not produce invertase is able to take advantage of and invade a population of wild-type cooperator cells. However, over a wide range of conditions, the wild-type cooperator can also invade a population of cheater cells. Therefore, we observe steady-state coexistence between the two strains in well-mixed culture resulting from the fact that rare strategies outperform common strategies—the defining features of what game theorists call the snowdrift game[17]. A model of the cooperative interaction incorporating nonlinear benefits explains the origin of this coexistence. We are able to alter the outcome of the competition by varying either the cost of cooperation or the glucose concentration in the media. Finally, we note that glucose repression of invertase expression in wild-type cells produces a strategy that is optimal for the snowdrift game—wild-type cells cooperate only when competing against cheater cells.

Yeast prefers to use the monosaccharides glucose and fructose as carbon sources. However, when these sugars are not available, yeast can metabolize alternative carbon sources such as the disaccharide sucrose[18]. After sucrose is hydrolysed by invertase, the resulting monosaccharides are imported[13,14], yet some of the glucose and fructose may diffuse away from the cell before it is able to import them into the cytoplasm (Supplementary Fig. 1). If such sugar loss by diffusion is significant then we might expect high-density cultures to grow more quickly than low-density cultures, because cells at high density benefit from their hydrolysis products and those of their abundant neighbours. Indeed, we find that cells grown in media supplemented with sucrose—but not glucose—grow much faster at high cell density than at low cell density. The growth rate at high cell density in 5% sucrose is similar to the growth rate at saturating (2%) glucose concentrations. However, the growth rate at low cell density is ~40% lower, equivalent to the growth rate in only 0.003% glucose (Supplementary Fig. 2). The fraction of invertase-created glucose that is captured can be estimated by dividing the rate of glucose uptake of cells growing in 0.003% glucose by the measured rate of invertase activity, yielding an estimated glucose capture efficiency of only ~1% (Supplementary Fig. 3). Analytic calculations of glucose diffusion suggest that this low capture efficiency is an expected consequence of diffusion and the known properties of the sugar importers (Supplementary Fig. 4).

Given that 99% of glucose created by a cell is lost to neighbouring cells, it may be possible for a 'cheater' strain to take advantage of the cooperators by not secreting invertase and instead simply consuming the glucose created by other cells[15]. If cooperative cells shared all of the glucose that they created (that is, if 100% of hydrolysed glucose and fructose diffused away from the hydrolysing cell), then both the cooperators and the cheaters would have the same access to sugar, yet only the cooperators would bear the metabolic cost of invertase production and secretion. In this case, the cheaters would always outgrow the cooperators, and the interaction would be what is called a prisoner's dilemma, in which cooperation is not sustainable in a well-mixed environment[1,17]. However, we found that yeast retains a small fraction of the glucose created by sucrose hydrolysis, which may be sufficient to allow cooperative strategies to survive.

To explore this problem, we performed a set of competition experiments between the wild-type strain ('cooperator') and a mutant strain lacking the invertase gene ('cheater' or 'defector'; see Supplementary Fig. 1). Consistent with there being a metabolic cost associated with invertase production, we find that in glucose-supplemented media, cooperators grow more slowly than cheaters only when invertase is being expressed (Supplementary Fig. 5)[15]. In addition, the cooperator strain in our experiments is a histidine auxotroph; therefore, limiting the histidine concentration in the media slows the growth of the cooperator relative to the cheater, allowing us to experimentally increase the 'cost of cooperation' (Supplementary Fig. 6). We can measure the relative abundance ('fractions') of the two strains in a mixed culture by flow cytometry because they express different fluorescent proteins (Supplementary Fig. 7).

We began by monitoring the change over time in the fractions of cooperators and cheaters co-cultured in sucrose media. Each co-culture started from a different initial fraction of cooperators, and each day we performed serial dilutions into fresh media and measured the cell density and relative abundance of the two strains. In cultures starting with a small fraction of cheaters, the cheaters increased in frequency, consistent with the cheaters 'taking advantage' of the cooperators (Fig. 1a). However, when the initial fraction of cooperators was low, we found that the frequency of cooperators increased, suggesting that in the steady state there will be coexistence between the two strains. Indeed, the equilibrium fraction is independent of the starting fraction but depends upon the histidine concentration (Fig. 1b; the equilibrium fraction in saturating histidine was $f \approx 0.3$). As the cost of cooperation increased, we observed a decrease in both the equilibrium fraction of cooperators and the mean growth rate of the culture at equilibrium (Fig. 1c). A large cost of cooperation therefore allows the cheaters to dominate the population but also results in a low growth rate of both strains. Coexistence was also observed in continuous culture, meaning that the 'seasonality'

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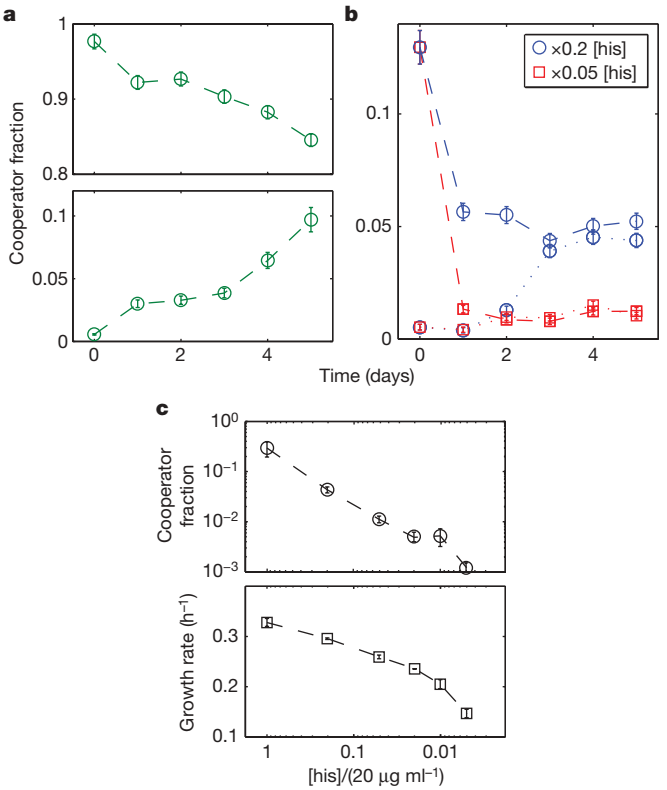

Figure 1 | Competition between the wild-type cooperator and mutant cheater strains. **a**, In sucrose culture, a small fraction of cheaters can invade a population of cooperators (top), and a small fraction of cooperators can also invade a population of cheaters (bottom), together implying coexistence between the two strains at steady state (histidine concentration ([his]), $20\,\mu g\,ml^{-1} \equiv \times 1$; no imposed cost of cooperation). **b**, As the histidine concentration becomes limiting we find that equilibrium between the two strains is reached within experimental timescales regardless of starting fractions. The fraction of cooperators at equilibrium does not depend upon the starting fraction but does depend upon the histidine concentration. **a** and **b** show typical data; error bars reflect sensitivity of measured fractions to different cut-off values (Supplementary Fig. 7). **c**, Both the equilibrium fraction of cooperators (circles) and the mean growth rate (squares) decrease as the cost of cooperation increases (lower histidine concentrations). Error bars, s.e.m.; $n = 3$.

imposed by serial dilution in batch culture is not necessary for coexistence[11] (Supplementary Fig. 8).

When the cooperators are initially only a small fraction of the population, then there will be little glucose available in the media. In this case, the cooperators have an advantage because they are able to capture at least some small fraction of the glucose that they create. As the fraction of cooperative cells increases, the glucose concentration also increases, and eventually the growth rates of the two strains become equal. Similarly, if the initial fraction of cooperative cells is above the equilibrium level, then their fraction will decrease; as this occurs, we find that the growth rate of the culture also decreases (Supplementary Fig. 9). Such a decrease in mean population fitness caused by evolutionary dynamics is a defining feature of the challenges posed by cooperation.

Our experimental observation of coexistence between the cooperator and cheater strains implies that the interaction is governed by what game theorists call the snowdrift game (also known as the hawk–dove game or the game of chicken)[3,17]. The snowdrift game derives its name from the potentially cooperative interaction present when two drivers are trapped behind a large pile of snow, and each driver must decide whether to clear a path. In this model of cooperation, the optimal strategy is the opposite of the opponent's (cooperate when your opponent defects and defect when your opponent cooperates). The snowdrift game is therefore qualitatively distinct from the prisoner's dilemma, in which all players have the incentive to cheat regardless of the strategies being followed by the others. Coexistence between cooperation and defection arises in a snowdrift game because rare strategies, which will often interact with the opposite strategy, do comparatively well.

To understand why sucrose metabolism is a snowdrift game, we constructed a simple phenomenological game theory model of the interaction. We assumed that invertase expression has a cost c and generates total benefits of unity that are captured with efficiency ε. In this scheme, for large capture efficiencies and/or small costs of cooperation ($\varepsilon > c$), the cooperators always outgrow the defectors and therefore take over the population (Fig. 2a). However, for small capture efficiencies and/or large costs ($\varepsilon < c$), the interaction is a prisoner's dilemma in which the defectors always do better, leading to extinction of the cooperators. However, in our experiments we observed coexistence between the two strains, an outcome that never occurs in the simple model of Fig. 2a. The ability to capture a sufficiently large fraction of the benefits of cooperation can allow cooperators to take over a population, but does not on its own lead to coexistence between cooperators and cheaters.

Coexistence of the two opposing strategies requires that the strains are mutually invasible. In particular, a lone cooperator must outperform a population composed entirely of defectors[17]. Indeed, we have already found experimentally that wild-type yeast in dilute cellular conditions is able to grow at a significant rate despite capturing only ~1% of the glucose created (Supplementary Fig. 2). This is because growth as a function of glucose is highly concave; doubling the glucose concentration therefore does not double the growth rate. By measuring the growth rate as a function of glucose concentration, we conclude that all benefit terms in our model should be raised to the power of $\alpha = 0.15 \pm 0.01$ (Supplementary Fig. 10 and Fig. 3c). Including this nonlinear effect alters the phase diagram and creates a large region of parameter space that is a snowdrift game in which there is coexistence between the two strategies[19] (Fig. 2b; $\alpha > 1$ leads to bistability[19,20] (Supplementary Table 1)). The saturating nature of growth on glucose means that a small number of cooperators can supply the glucose for many cells, thus providing a natural explanation for the small fraction of cooperators often observed in our competition experiments (Figs 1c and 3a, b).

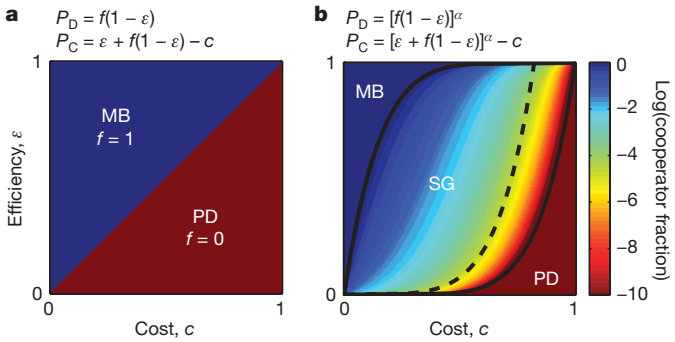

Figure 2 | Game theory models of cooperation in sucrose metabolism. **a**, Defection and cooperation payouts, respectively P_D and P_C, and the resulting phase diagram of the cooperative fraction, f, at equilibrium in a simple linear model in which cooperation has a cost c and leads to total benefits of unity that are captured with an efficiency ε. This model leads to fixation of cooperators ($f = 1$) at low cost and/or high efficiency of capture ($\varepsilon > c$, implying that the game is mutually beneficial (MB)[5]) but fixation of defectors ($f = 0$) for high cost and/or low efficiency of capture ($\varepsilon < c$, implying that the game is prisoner's dilemma (PD)). **b**, A model of cooperation with experimentally measured concave benefits yields a central region of parameter space that is a snowdrift game (SG), thus explaining the coexistence that is observed experimentally ($\alpha = 0.15$ in figure; see Supplementary Fig. 10). Adding glucose makes the cheaters less reliant on the cooperators, thus reducing the range of parameters in which cooperation can survive (solid to dashed line; see Supplementary Fig. 11).

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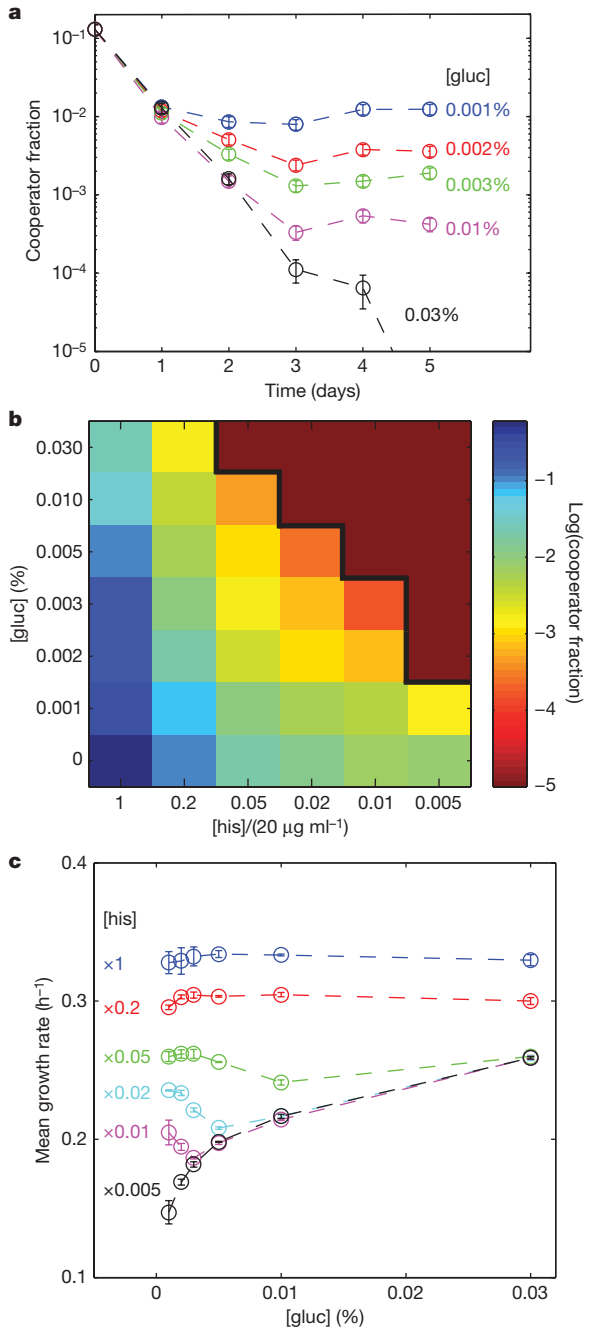

Figure 3 | Varying the glucose concentration can transform the outcome of competition. **a**, As the glucose concentration ([gluc]) in the media increases, the equilibrium fraction of cooperators decreases ([his] = ×0.05 = 1 μg ml^{-1}). Typical data shown; error bars reflect sensitivity of measured fractions to different cut-off values (Supplementary Fig. 7). **b**, Fraction of cooperators at equilibrium as a function of the glucose and histidine concentrations (all cultures have 5% sucrose; mean of two or three independent experiments; see Supplementary Fig. 12 for errors). The cooperators can be driven to extinction by either increasing the cost of cooperation or adding glucose to the media (solid black line denotes the extinction boundary). **c**, Mean growth rate of co-culture at equilibrium as a function of glucose concentration. Error bars, s.e.m.; $n = 3$. Adding glucose can decrease the growth rate at equilibrium because there are fewer cooperators to hydrolyse sucrose. As expected, if there are no cooperators at equilibrium then the growth rate is not a function of the histidine concentration. The nonlinear relationship between growth rate and glucose concentration is visible in the ×0.005 [his] data (black).

The sublinear relationship between growth rate and glucose suggests that the glucose concentration in the media may be an important parameter governing the cooperative interaction. As the glucose concentration increases, the cheaters become less reliant on the cooperators, and cooperation becomes more difficult to maintain (dashed line in Fig. 2b and Supplementary Fig. 11). Therefore, we expect that adding glucose will decrease the fraction of cooperators at equilibrium, eventually transforming the game into a prisoner's dilemma and driving the cooperators to extinction. The glucose concentration necessary to transform the game into a prisoner's dilemma is expected to be a decreasing function of the cost of cooperation. These predictions and the associated phase diagram can be confirmed experimentally (Fig. 3a, b and Supplementary Fig. 12).

Increasing the amount of glucose available in the media decreases the fraction of cooperators at equilibrium and can even drive the cooperators to extinction. As the cooperators decrease in frequency, the amount of sucrose being hydrolysed also decreases. We find that for some costs of cooperation, this effect is so severe that the equilibrium growth rate of the mixed culture actually decreases as we add glucose to the media (Fig. 3c). This non-intuitive decrease in the co-culture growth rate is a striking result of the cooperative interaction, as the growth rate of each strain cultured alone increases as glucose levels increase in the media.

Similar to many other alternative modes of carbon metabolism, invertase expression is repressed at high concentrations of glucose[18]. Given this genetically encoded strategy, we can ask how a wild-type cell responds when placed in competition against cells that either always cooperate or always defect. Competition against always-defecting cells leads to low glucose concentrations, resulting in wild-type cells cooperating by expressing invertase (as in our competition experiments). By contrast, a wild-type cell competing against an always-cooperating strain would result in the glucose concentration rising to the point (>0.1%) at which invertase expression is repressed, thus causing the wild-type cell to cheat[18,21] (Supplementary Fig. 5a). We therefore see that the wild-type invertase-production strategy is exactly what might be expected in a snowdrift game—wild-type cells pursue the strategy opposite to that of their opponents. It is possible that glucose repression of invertase is partly determined by these social considerations, helping to make a population of wild-type cells relatively immune to invasion by strains with alternative strategies[22].

Our results are consistent with a recent study which found that a cheater strain was more fit than the wild-type cooperator strain when growing at high density on a sucrose plate[15]. In that paper, sucrose metabolism was classified as a prisoner's dilemma, although the experimental results are also consistent with the cooperative interaction being a snowdrift game. Distinguishing between these two games requires observation of competition at low starting fraction of cooperator. In addition, the competition must be performed in a well-mixed environment because spatial structure, such as the agar plate used in ref. 15, can drastically affect the outcome of competition[16,23].

The experimental observation of coexistence between cooperator and cheater strains in a well-mixed environment makes sucrose metabolism in yeast a particularly clear example of the snowdrift game[24], and may explain the existence in wild yeast populations of copy number variation in the *SUC2* gene, including the presence of cheaters[25]. Coexistence between cooperator and cheater strains in our experiments provide a concrete example of how interactions between alternative alleles can promote biological diversity[11,24,26]. Similar cooperative interactions may be present in other enzymatic processes that occur in the periplasmic space of yeast such as phosphate scavenging, starch degradation and phospholipase activity. It would be interesting to study the outcome of competition between the cooperator and cheater strains in spatially structured environments[9,15,16,23,27–29], particularly given a recent theoretical prediction that spatial structure often inhibits cooperation in a snowdrift game[27].

METHODS SUMMARY

Strains. All strains were derived from haploid cells BY4741 (mating type **a**, EUROSCARF). The 'wild-type' cooperator strain has an intact *SUC2* gene, a

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defective *HIS3* gene (*his3Δ1*) and yellow fluorescent protein expressed constitutively by the *ADH1* promoter (inserted using plasmid pRS401 containing *MET17*). The mutant cheater strain lacks the *SUC2* gene (EUROSCARF Y02321, *SUC2::kanMX4*), has an intact *HIS3* gene, and has the fluorescent protein tdTomato expressed constitutively by the *PGK1* promoter (inserted using plasmid pRS301 containing *HIS3*). Growth rate and invertase expression experiments in Supplementary Figs 2 and 5a were done using a strain containing yellow fluorescent protein driven by the *SUC2* promoter (inserted using plasmid pRS306 containing *URA3*).

Competition experiments. Co-culture experiments were performed in 5 ml batch culture at 30 °C using synthetic media (minus histidine) supplemented with 5% sucrose and variable concentrations of glucose and histidine. Cultures were maintained in a 'well-mixed' condition by growing in an incubator shaker at 225 r.p.m. The 20% sucrose stock solution was filter-sterilized and stored with 1 mM Tris buffer, pH 8.0, to prevent acid-catalysed autohydrolysis. Nevertheless, 5% sucrose media typically had a monosaccharide concentration of ~0.0001%. The experiments described in Fig. 1 have 0.001% glucose added manually. Serial dilutions were performed daily (23 h of growth) such that the starting optical density was 0.0025, corresponding to ~150,000 cells. Fractions were determined using a BD FACScan flow cytometer (Supplementary Fig. 7) and periodically confirmed by selective plating. Equilibrium data in Figs 1c and 3b, c were recorded after five days of competition between the two strains.

Received 23 November 2008; accepted 11 February 2009.
Published online 6 April 2009.

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

Acknowledgements The authors would like to thank D. Kim, A. Raj, K. Gora, D. Muzzey and B. Pando for discussions and/or experimental help. This work was supported by grants from the US National Institutes of Health (NIH) and National Science Foundation to A.v.O. J.G. is supported through a Pappalardo Postdoctoral Fellowship and an NIH K99 Pathways to Independence Award. H.Y. was supported by a Lester Wolfe Fellowship.

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