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The Prisoner's Dilemma and polymorphism in yeast *SUC* genes

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The *SUC* multigene family of the single-celled yeast *Saccharomyces cerevisiae* is polymorphic, with genes varying both in number and activity. All of the genes encode invertase, an enzyme that is secreted to digest sucrose outside of the cell. This communal endeavour creates the potential for individual cells to defect (cheat) by stealing the sugar digested by their neighbours without contributing the enzyme themselves. We measured the fitness of a defector, with a deleted *suc2* gene, relative to an otherwise isogenic cooperator, with a functional *SUC2* gene. We manipulated the level of social interaction within the community by varying the population density and found that the defector is less fit than the cooperator at low levels of sociality but more fit in dense communities. We propose that selection for anti-social cheating causes *SUC* polymorphism in nature. The infamous Prisoner's Dilemma game shows that social behaviour is generally unstable, and the success of both cooperation and defection can vary continuously in time and space. The variation in *SUC* genes reflects constant adaptation to an ever-changing biotic environment that is a consequence of the instability of cooperation. It is interesting that social interactions can have a direct effect on molecular evolution, even in an organism as simple as yeast.

Keywords: *Saccharomyces*; SUC; multigene family; Prisoner's Dilemma; cooperation; defection

1. INTRODUCTION

The everyday problems of conflict and lack of cooperation are familiar to all of us, so it is not surprising that the evolution of cooperative behaviour is hard to fathom. The Prisoner's Dilemma game is a popular metaphor that clearly represents the difficulty (Axelrod 1984). Two players each choose whether to cooperate with the other player or to defect (cheat). Cooperation gives the greatest average advantage but unilateral defection gives the greatest individual advantage, to the defector. If the game is played only once defection is the superior strategy since it is the only strategy that can win, rather than tie or lose. However, in biologically realistic simulations, with repeated interactions, mutations, errors, or a spatially structured environment, there is no single stable solution and the success of individual strategies can vary in endless periodic or chaotic cycles (Nowak 1989; Nowak and May 1992; Nowak and Sigmund 1993; for a review see Brembs 1996).

The *SUC* genes of *Saccharomyces cerevisiae* are highly polymorphic. Strains vary both in the number of *SUC* genes that they possess near their telomeres (chromosome ends) and in whether a single non-telomeric gene (*SUC2*) is functional or a pseudogene (Carlson & Botstein 1983). Such wide variation is unusual and reminiscent of that seen in antigen or virulence genes of microbes as they evolve under the changing selective pressure of their hosts' immune systems (for a review see Moxon *et al.* 1994). However, *S. cerevisiae* is normally free-living, and the *SUC* genes do not encode an antigen but an enzyme: invertase. Unlike most other yeast enzymes, invertase is secreted outside of the cell, where it hydrolyses sucrose. This creates the potential for a Prisoner's Dilemma that can explain the polymorphism.

A yeast cell could defect by exploiting the enzyme produced by a neighbour whilst contributing little or none itself. The average fitness of a group would be highest when each member secretes an optimal amount of enzyme into the shared pool, but the fitness of an individual would be maximized by taking from the pool without bearing the cost of contributing. This social instability could drive molecular evolution if cooperation and defection were heritable behaviours. We tested this hypothesis experimentally.

2. MATERIAL AND METHODS

We measured the fitness of a defector relative to a cooperator as they competed in a structured environment, a sucrose-rich agar plate. We manipulated the role of sociality by inoculating plates with a wide range of cell numbers—from a few well-separated individuals to a dense layer of touching cells. The defector was a diploid strain supplied by Research Genetics (Invitrogen, Carlsbad, CA 92008, USA) in which both homologous copies of the *SUC2* gene were deleted and replaced with the selectively neutral marker *KanMX4*. By crossing the defector to an isogenic strain that had an intact *SUC*, dissecting tetrads and mating haploids with the appropriate genotypes, a cooperator diploid was produced that was genetically identical to the defector except that it had both wild-type copies of *SUC2*, and did not contain the marker *KanMX4*.

Initially, the two strains were grown separately to an equal density in 5 ml of liquid YEPD (yeast extract 1%, peptone 2%, glucose 2%), then mixed together in equal volumes and a 111 µl sample taken. The sample was serially diluted in five tenfold increments (1 in 10^5 overall) and then plated onto solid YEPD to produce single colonies that would be counted to determine the initial cell density. The initial frequency of the defector was also calculated from this sample by replica-plating the colonies onto YEPD that had been supplemented with 200 mg l^{-1} of G418, permitting only the *KanMX4*-containing defector colonies to grow. The mixed culture was also plated on solid sucrose plates (yeast extract 1%, peptone 2%, sucrose 2%, agar 2.5%) at a wide range of initial densities ranging from 1.6×10^9 down to 108 cells per plate. These fitness assay plates were incubated for three days at 30 °C. The colonies were then washed off in 10 ml of sterile water and mixed thoroughly by vortex machine for 30 s. The final cell numbers on each sucrose plate were determined by taking a 111 µl sample of the washed-off cells, serially diluting it, plating on YEPD plates and counting the resulting colonies as before. The final frequencies of the defector in each fitness assay population was calculated by replica-plating these colonies to YEPD supplemented with G418 and counting the G418-resistant defector colonies, as before. The YEPD plates and their G418 replicas were randomized and labelled by an assistant, so that the colony counts were performed blind. The relative fitness of the defector at each initial density was then calculated as the ratio of the Malthusian parameters (Lenski *et al.* 1991). Three replicate fitness assays were done at each of eight different levels of sociality. In addition, a control experiment was performed in which YEPD (glucose) plates were used instead of sucrose plates, with threefold replication, at the highest and lowest levels of sociality.

3. RESULTS

The results are shown in figure 1. A linear regression is highly significant ($r = 0.847$, 22 d.f., $p < 10^{-6}$). The

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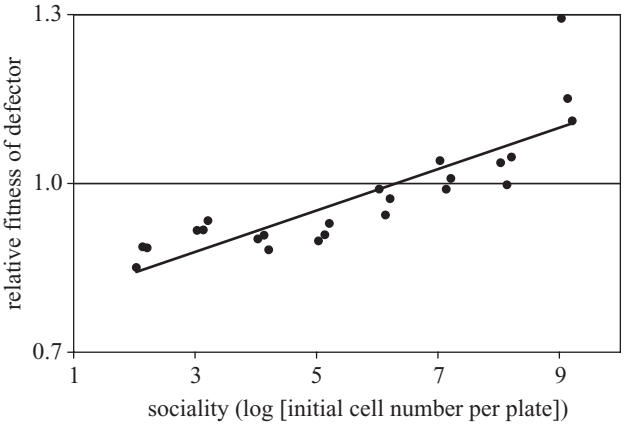

Figure 1. The relative fitness of the defector increases with sociality.

mean fitness of the defector relative to the cooperator is low (0.87) at the lowest cell density but high (1.19) in the densest population, a highly significant difference ($t = 5.49$, 4 d.f., one-tailed test, $p = 0.0027$). The control experiment confirmed that this was due to competition for sucrose: when glucose plates were used instead of sucrose the relative fitness of the defector did not differ significantly between the lowest (1.00) and highest (0.98) levels of sociality ($t = 0.778$, 4 d.f., one-tailed test, $p = 0.23$). Defection is therefore selected in dense populations of cooperators.

4. DISCUSSION

The unusual structure of the *SUC* gene family was previously thought to result from strong artificial selection for improved growth in certain domesticated strains, causing subtelomeric gene amplification (Naumov *et al.* 1996; Pryde & Louis 1997). However, although beer is produced from a maltose (not a sucrose) medium, brewing strains can contain many functional *SUC* genes (Denayrolles *et al.* 1997) as can the closely related species *S. paradoxus*, which is wild, i.e. non-domesticated (Naumov 1969). We suggest, instead, that the polymorphism in *SUC* genes reflects constant adaptation to a changing biotic environment that is a consequence of the instability of cooperation. Although our experiment tested only two discrete strategies, cooperation and defection, continuously variable heritable levels of enzyme production may be envisaged in real life. The subtelomeric region allows expansion and contraction of gene copy number (Pryde & Louis 1997) and could allow optimization of invertase expression, if a stable optimum existed. However, stable solutions to such a continuous Prisoner's Dilemma are as elusive and controversial as those to the original discrete game (e.g. Roberts & Sheratt 1998; Killingback & Doebeli 1999).

Genes at the telomeres have another unusual property in addition to copy-number instability: the phenomenon known as 'silencing'. The expression of subtelomeric genes is repressed at random and this silenced state is inherited for approximately 15 generations before normal expression resumes (Louis 1995). Our results indicate that defectors should invade closely cooperating populations, causing the mean fitness of such communities to decline. Eventually extinction or dispersal of these failed societies would allow the founding of new cooperative groups and natural selection would once again favour individuals able to utilize sucrose. Silencing might allow defectors to maintain an unused 'backup' copy of a *SUC* gene that can be restored at such a time.

SUC may not be the only multigene family to have evolved to respond to unstable social pressures. We note that several other polymorphic or subtelomeric multigene families of *S. cerevisiae*, such as *STA*, *FLO*, *PHO* and *MEL* (Jones *et al.* 1992) also encode extracellular proteins. Our results suggest that social interactions can have a direct effect on molecular evolution, even in the simplest of organisms.

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