Sociobiology of the budding yeast

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Social theory has provided a useful framework for research with microorganisms. Here I describe the advantages and possible risks of using a well-known model organism, the unicellular yeast *Saccharomyces cerevisiae*, for sociobiological research. I discuss the problems connected with clear classification of yeast behaviour based on the fitness-based Hamilton paradigm. Relevant traits include different types of communities, production of flocculins, invertase and toxins, and the presence of apoptosis.

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1. Introduction

For many years sociobiological research had focused on large eukaryotes, including humans, and the fascinating social insects (Wilson 1978). Nowadays there are more and more papers in which behaviour is analysed from the perspective of microbes. Sociobiology was defined by EO Wilson as 'The extension of population biology and evolutionary theory to social organization'. The term 'social' refers to characteristics of living organisms that interact with each other, and to their collective coexistence, irrespective of whether they are aware of it or interact voluntarily. The term 'behaviour' can be defined as the actions performed by organisms, systems or artificial entities in conjunction with their environment, which includes other systems or organisms as well as the physical environment. 'Behaviour' is thus the response of the system or organism to various stimuli or inputs, whether internal or external, conscious or subconscious, overt or covert, and voluntary or involuntary (Hamilton 1964a, b). If we agree with these definitions, then there is no reason to exclude yeast from research endeavours into social biology.

The organisms taking part in social behaviours can be divided into actors, that is, those who perform the behaviour, and recipients, those who experience the results of that the action. According to Hamilton's fitness-based classifications, it is possible to distinguish the following behaviors: 'mutually beneficial', a behavior that increases the *direct fitness* (see glossary) of both the actor and the recipient; its opposite, 'spite', when both players' fitness is reduced; 'selfish', a situation where the actor gains while the recipient suffers a loss; and 'altruistic', when the recipient benefits but the actor's fitness is reduced (Hamilton 1964a, b; West *et al.* 2006; Diggle 2010). Finally, the behaviour is called 'cooperation' when the fitness of the recipient is enhanced, irrespective of the fate of the actor (figure 1). Costs and benefits are defined in terms of the lifetime reproductive success of the biological entity, which in this article is the yeast cell.

In this review I present and discuss examples of yeast behaviour that have been classified as social. This includes community organization and invertase production understood as a form of cooperation, toxin production by killer phenotypes as a case of selfishness, and apoptosis as a yet to be proved as social behaviour.

2. Yeast as a universal model organism

Using model organisms in research provides an opportunity to understand universal mechanisms. Moreover, the results may serve as a reference for other organisms (including humans), on which research is too complicated or unethical to perform. This assumption is validated by the common origin of all organisms, the conservatism of metabolic processes and pathways, as well as the similarities in the genetic material and in methods of inheritance.

Keywords. Apoptosis; colony formation; cooperation; *Saccharomyces cerevisiae*; sociomicrobiology; toxins

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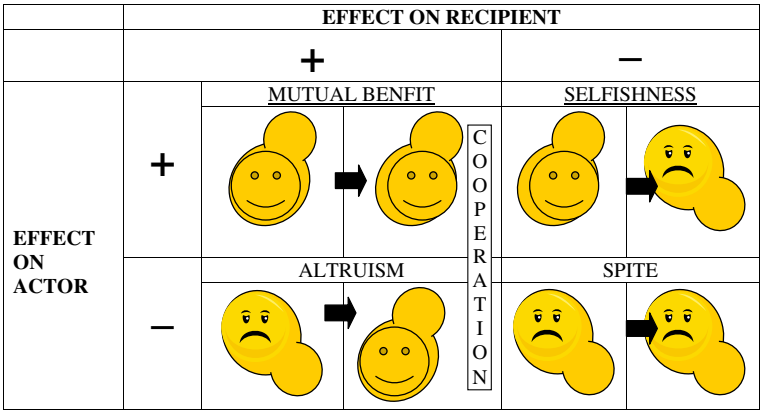

Figure 1. Classification of social behaviour (based on other reviews: West *et al.* 2006; Diggle 2010).

The eukaryotic unicellular fungus *Saccharomyces cerevisiae* is one of the most important and extensively studied model organisms, with a long and distinguished experimental history (Forsburg 1999, 2001). Because of its ease of genetic manipulation, laboratory handling and long-term storage, it has found wide applications in all types of biological research. (Here are examples of the research of our group: Wloch *et al.* 2001; Szafraniec *et al.* 2003; Bobula *et al.* 2006; Jasnos and Korona 2007; Tomala *et al.* 2011; Jakubowska and Korona 2012). Among eukaryotes, it was the first to be transformed by plasmids (Beggs 1978), the first to experience gene-targeting (Rothstein 1983) and the first whose genome was completely sequenced (Goffeau *et al.* 1996). As stated in a recent comprehensive review, yeast is still the most facile organism for studying the relationship of genotype to phenotype in eukaryotic cells. Finally, a very useful resource for the field can be found in the *Saccharomyces* Genome Database (*www.yeastgenome.org*), where the combined efforts of the worldwide yeast research community are gathered in a constantly updated and freely accessible form.

Because of its long-standing history as a domesticated organism, most of our knowledge of *S. cerevisiae* comes from strains present in the laboratory, vineyard or brewing environment (Fay and Benavides 2005; Liti *et al.* 2009); little is known about the ecology and population structure of this species in nature (Liti *et al.* 2009). The occasional isolates found in nature were thought to be feral strains that originated from domestic stocks (Naumov *et al.* 1996, 1998; Buzzini and Martini 2000; Goddard *et al.* 2010; Zhang *et al.* 2010). The identification of wild *S. cerevisiae* from oak trees in Siberia and North America (Naumov and Naumova 1991; Naumov *et al.* 1998) suggested that *S. cerevisiae* is not entirely a human commensal species. Subsequent population genetic studies showed that wild oak tree populations are differentiated from those associated with humans (Fay and Benavides 2005; Liti *et al.* 2009; Schacherer *et al.* 2009; Liti and Schacherer 2011). In a recent study, Wang *et al.* (2012) present results of genetic analysis of thousands of samples collected from diverse arboreal habitats across China. Environments listed include: fruit, bark, soil and rotten wood of primeval forests undisturbed by humans, secondary forests, planted orchards and urban trees in both tropical and temperate regions. Genetic analysis of 99 *S. cerevisiae* isolates revealed 9 genetically distinct groups, 5 of which are basal to all previously defined groups, including that from North American oak trees. Interestingly, the three groups that fell within previously described populations were all isolated from secondary forests and orchards (Wang, *et al.* 2012). Such studies give hope that in the near future we will substantially expand our knowledge of the diversity, ecology, evolution, domestication history and even sociobiology of this wild yeast (Fay 2012). However, for now, most of the considerations presented in this article must be based on the results of laboratory experiments mainly dealing with single characterized strain, besides which the studies have been often restricted to clonal cultures (Botstein and Fink 2011).

3. Organization of yeast communities

Saccharomyces cerevisiae is mostly thought of as a solitary, unicellular species (for example, Madigan *et al.* 2009). However, this is an oversimplification. In the laboratory, yeast cells exist so rarely in a truly solitary state that solitariness cannot be considered a hallmark of this species. Soon after sensing an adequate environment, a single healthy yeast cell, haploid or diploid, will start to divide; what results is a whole new clonal population in which the density can reach up to the ~ 2×10^8 cells/mL in the laboratory conditions. There is no data about the density of the populations existing in nature.

Incomplete separation of the budded daughter cells can result in the presence of aggregates, which in most feral

S. cerevisiae strains usually consist of 6–10 clonal cells (Wloch-Salamon, unpublished results). Stains that do not form aggregates were deliberately constructed and chosen for laboratory use during the early years of yeast research (Mortimer and Johnston 1986), and today's widely used laboratory strains, S288c and W303, are derived from those efforts.

A second type of cell clumping, distinct from aggregation, is seen in yeast and is called flocculation. This clumping process has been mostly ignored by scientists because laboratory strains do not flocculate (Mortimer and Johnston 1986). However, many strains used in brewing form large clumps, called 'flocs', which makes them easier to remove from beer once fermentation is complete (it is thought that the flocculation trait was selected for by brewers for this reason). Flocculation is different from the aggregation mentioned earlier and its effect can be reversed by adding ETDA to the culture (Smukalla *et al.* 2008; Bruckner and Mosch 2012). In contrast, adding ETDA does not influence the presence of aggregates in an aggregating strain. Yeast flocculation is regulated by adhesin proteins, which in *S. cerevisiae* are also termed 'flocculins'. To date, several different flocculins have been identified in diverse industrial and laboratory strains that confer vegetative adhesion: *FLO1, FLO5, FLO9, FLO10, FLO11* (or *MUC1*) and *AGA1* (Soares 2011). Flocculins are initially fixed to the cell wall by a glycosylphosphatidylinositol (GPI) anchor near the C terminus and require a lectin-like N-terminal domain to bind oligosaccharides on neighbouring cells. Flocculating cells also produce a mixture of polysaccharides around the exterior of the cell, called the extracellular matrix (ECM). Production of the ECM facilitates the formation of pores for water and nutrient flow that protect communities against dehydration (Flemming and Wingender 2010), but blocks the permeation of large toxic molecules into the cell (Douglas 2003; Kuthan *et al.* 2003). Given that the extracellular material isolated from colonies possesses a high water retention capacity, the ECM may also be involved in the storage of water and possibly nutrients. Finally, flocculins are necessary to form elongated cell chains called pseudohyphae, which help the colony to anchor itself to the surface (Vachova *et al.* 2011). ECM and *FLO11* expression is proved to be regulated via quorum sensing mechanism (see glossary). *S. cerevisiae* uses ethanol and the aromatic alcohol tryptophol and phenylethanol as autoinducers in a cell density-dependent manner (Chen and Fink 2006). When the cell density is sufficiently high, the production of ethanol and aromatic alcohols reaches a threshold, activating *FLO11* expression via the PKA pathway (Chen and Fink 2006; Bojsen, *et al.* 2012). Hence, tryptophol and phenylethanol possibly influence *S. cerevisiae* biofilm development through the regulation of *FLO* genes.

Flocculating vs non-flocculating strains of yeast demonstrate differing growth morphologies on liquid and solid media (figure 2). On solid substrates exposed to air, cells that do not produce flocculins will develop nonadhesive colonies, such as seen for the laboratory strain S288c (figure 2B), and is the typical growth form of many laboratory strains on solid agar media. When expressing genes for

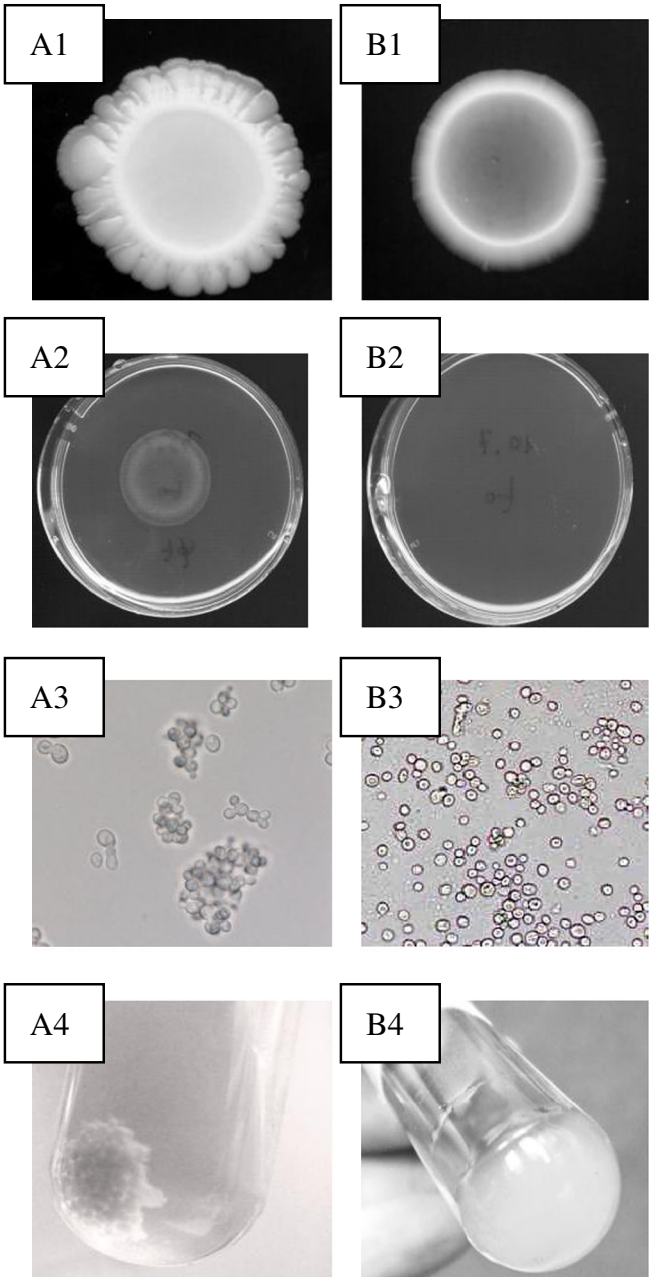

Figure 2. Examples of different phenotypes of (**A**) feral yeast and (**B**) laboratory (s288c) on: (**1**) YPD agar plates (diameter of the colonies are ~1 cm); (**2**) colony washed from the 10 mL plate, diameter ~6 cm; (**3**) under microscope (magnitude 400×); (**4**) in liquid YPD, 10 mL vials. Photos by Katarzyna Pawlik (**A3**–**B4**) and the author (**A2**–**B2**).

cell–cell adhesion (*FLO1*, *FLO5*, *FLO9*, *FLO10*), yeast cells can form non-dissolvable colonies of cells that stay closely together (figure 2A). In order to develop colonies that are not removable from the surface, adhesins that confer for this trait (*FLO10* and *FLO11*) must be produced. Cell-to-cell and cell-to-surface adherence is necessary for biofilm and invasive filaments production (Honigberg 2011; Bruckner and Mosch 2012). In a liquid medium, nonadhesive yeast cells are planktonic and produce turbid cultures of individual cells (as do the laboratory strains). When producing proteins for self-adhesion (flocculins), yeast cells can form aggregates that may sediment to the bottom (flocs) or that can float on the liquid surface (flor), biofilms and filaments (figure 2) (Honigberg 2011; Bruckner and Mosch 2012).

Because of the technical difficulties of examining the interiors of yeast multicellular structures, flocs or colonies, there has been, until recently, little study on colony development (Bojsen *et al.* 2012). Colony formation in yeast involves spatiotemporal localization of specific cell subpopulations with different functions (Vachova *et al.* 2011; Stovicek *et al.* 2012). After a few initial cell divisions on an agar plate, particular cell subpopulations begin to diverge and adopt distinct roles. Cells at the colony base form pseudohyphae (see glossary). These filaments invade the agar medium, anchoring the structure to the solid substrate. Cells in peripheral layers surrounding the entire colony (including subsurface parts) are equipped with drug-efflux pumps (expressing the genes for protein Pdr5p and Snq2p). These proteins belong to the family of pleiotropic drug resistance membrane transporters and are capable of removing various (including toxic) substances from the cells and protecting them (and thus also the whole colony) against external attacks (Vachova *et al.* 2011; Stovicek *et al.* 2012). In addition to the presence of these pumps, cells at the surface layers of the aerial portion of the colony enter a stationary phase of growth and thus become more resistant to potential environmental stress. Meanwhile, cells in the interior of the colony produce an extracellular polymeric matrix (ECM) as described above. While the observations described above were made on laboratory strains of yeast, the architecture of colonies formed by various wild *S. cerevisiae* strains has been found to be comparable, indicating that colony formation is probably similar among all *Saccharomyces* species (Vachova *et al.* 2011; Bojsen *et al.* 2012; Stovicek *et al.* 2012). However, the recent discovery of many new arboreal, non-human-associated *S. cerevisiae* isolates (Wang, *et al.* 2012) indicates that there may be a wider array of phenotypic diversity among yeasts than previously known possibly including novel colony morphologies (Fay 2012).

The actual benefits and costs of labour division experienced by specific types of cells during colony formation were not evaluated (Vachova *et al.* 2012). However, some insights are given in an older study (Palkova and Vachova 2006; Smukalla *et al.* 2008). *S. cerevisiae* cells with and without *FLO1*$^+$ expression were subjected to various stress treatments, after which the percentage of surviving cells was determined. Results shows that colony of *FLO1*$^+$ cells tolerate about twice as much alcohol and more than 100 times the concentration of antifungal drugs than when there are as solitary cells. However, the cells at the colony centre were shielded not only from toxic effects, but from nutrients and oxygen as well. When flocculating and non-flocculating strains were grown in competitions in the optimal conditions, the outcome has shown that the *FLO1*$^+$ populations slowed growth rate more than 4-fold as compared to the non-flocculating strain. Even where the *FLO1*$^+$ strains are chemically prevented from clumping together and making the flocs (in medium containing mannose), the active *FLO1* makes them grow more slowly. This reduced fitness represents pure metabolic costs of *FLO1* expression (Smukalla *et al.* 2008). So now we face the common dilemma expressed by evolutionary biologists: *Why are communities of cooperating individuals not torn apart and taken over by 'cheaters', who reap communal benefits while contributing nothing?* One possible explanation stems from the fact that monotype population growth ensures that offspring are essentially clonal, and thus a reduced fitness associated with cooperation could be tolerated because of benefits shared among kin. The price associated with expression of *FLO1* provides an explanation for why not all wild strains switch it on, but it also raises the question of why flocs are not invaded by cheater strains. The answer is that, for the most part, cheater strains are physically prevented from invading flocs. Smukalla mixed cells with and without active *FLO1* expression in equal measure and left them to mingle for many hours: by the end of the incubation the cells with active *FLO1* had almost entirely congregated in flocs. Some cheating *FLO1-less* cells managed to find their way within the floc, which is not surprising given that they soon outnumber the *FLO1* peers with their speedier growth rate. But even so, the *FLO1* cells have such an affinity for each other that they push many of the cheaters into the outer layer of the flock, where they end up as the first line of defence for the rest of the community (Smukalla *et al.* 2008). The other hypothesis is that the *FLO1* gene might be a rare case of a 'green beard gene' (see glossary) (Hamilton 1964a, b). This assumes the presence of a pleiotropic gene that encodes for a certain trait, advantageous to its bearer (related to cooperation), together with a distinguishing phenotype (so called 'green beard'). Green beard allows cooperating individuals to recognize each other in the mixed population and cooperate without a risk of cheating. Such identification allows for directed cooperating behaviour only with other cooperating individuals. In the *FLO1* example, the same gene codes for both: flocculin production, enabling recognition and so leading to adhsesion to other cooperators, and for physical rejection of non-cooperators (non-flocculating individuals). Another requirement of a true green beard gene is that such behaviour should be manifested irrespective of genetic relatedness. Smukalla transferred the *FLO1* gene from *S. cerevisiae* to another closely related species of yeast, *Saccharomyces paradoxus*, which has no *FLO1* version of its own and in its natural condition does not flocculate. Amazingly, the addition of this single gene gave *S. paradoxus*

the ability to form flocs and when the two species were jumbled, they formed mixed-species flocs. Current findings on variability in *FLO* genes has led to suggestions that subtly different versions of *FLO* might allow natural yeast populations to discriminate among one another (Van Mulders *et al.* 2009, 2010), although this has not yet been demonstrated. Our increasing knowledge of phylogeny and population genetics on a large set of naturally isolated strains (Wang *et al.* 2012) could be a good source of material to check if there may be many different 'beard colours' in nature.

3.1 *Invertase secretion – altruism or kin selection?*

Yeasts secrete a number of enzymes, including acid phosphatase, phospholipase and invertase, that release utilizable nutrients from precursor molecules in the external medium. In the example of invertase, the secreted enzyme breaks down an otherwise inaccessible source of energy, the disaccharide sucrose in the external medium, into the monosaccharides glucose and fructose, which can then be imported into the cell by hexose transporters. These sugars can also 'escape' into the medium by diffusion away from the cell (Dodyk and Rothstein 1964). Extracellular hydrolysis of sucrose thus allows other cells to share glucose and fructose. This production is not regulated by a quorum-sensing mechanism, which is dependent on the local density of the cells, but is an outcome of the regular physiology of the producer cell. Because invertase is often treated as a secreted 'public good' (see glossary), it has been used to investigate social interactions (Greig and Travisano 2004; Koschwanez *et al.* 2011). Cells that do not make invertase are often referred to as 'cheaters' since they can grow on the monosaccharides that are liberated by invertase-producing cells without paying the cost of production. Cells that produce invertase incur a fitness cost, which was measured to be a 0.35% lower growth rate for cells that are forced to express invertase when grown in 1 mM glucose (Koschwanez *et al.* 2011). When a mixture of invertase non-producing (*SUC2*Δ) and invertase-producing (*SUC2)* cells is inoculated together on plates, the fate of each specific population depends on its initial density. At low densities, the ratio of *SUC2* to *SUC2*Δ cells increases because the cells that cannot make invertase are too far apart from those that can to profit from the diffused sugars. But at high densities, *SUC2*Δ cells outgrow *SUC2* cells, presumably because they have access to the sugars and do not have to bear the expense of producing invertase. However, their dominance finishes as soon as the *SUC2* cells die out, and so the source of accessible energy dries up (Greig and Travisano 2004).

Until now there is no known mechanism that allows the 'targeting' of the production of monosaccharides that will reach the producer cell or the relative. Therefore, production of invertase could be classified as altruistic cooperation lowering the fitness of the producers, and allowing growth of both: other SUC2 and *SUC2*Δ cells. However, in the case of colony growth on agar plates, or population-forming flocs (or aggregates) in non-shaken liquid medium, we can presume that most of the produced sugars reach relatives who are nearby. In such a case this whole story could be understood as an example of *kin selection* (see glossary), which favours relatives over non-relatives. The authors of the recent papers go even a bit further with their hypothesis stating that 'Since the evolution of secreted enzymes predates the origin of multicellularity, we argue that the social benefits conferred by secreted enzymes were the driving force for the evolution of cell clumps that were the first, primitive form of multicellular life' (Koschwanez *et al.* 2011). The most frequently occurring sugars in the natural environments are monosaccharides, glucose and fructose (composing a disaccharide, sucrose). So, it would be valuable to confirm the role of this mechanism for the 'public goods' other than sucrose.

3.2 *Toxin production – selfishness or spite? What is the role of the co-evolved viral particle?*

Toxin production is a widespread phenomenon within living organisms. It has been seen in bacteria, sponges, paramecium (Sonneborn 1943), social amoebae (Mizutani*, et al.* 1990) yeast (Bevan and Woods 1966) and other fungi. Analysis of *E. coli* strains collections (Riley and Gordon 1999; Gordon and O'Brien 2006) suggest that at least 35% of the strains could produce toxins. This number could even be an underestimate because of the problems related to the isolation of all bacterial strains and species present in different environments. Toxin producers, so called 'killer' strains, are also found among numerous yeast genera, for example, *Candida, Cryptococcus, Debaryomyces, Kluyveromyces, Pichia, Ustilago, Torulopsis* and *Saccharomyces*. Estimates of killer activity among wild yeasts from various habitats suggest that between 5% and 30% of the strains produce toxins that can kill a standard sensitive *Candida glabrata* strain (Starmer *et al.* 1987, 1992; Gulbiniene *et al.* 2004). Production of yeast toxins is associated with the presence of cytoplasmically inherited satellite dsRNA viruses of two types: ScV-M; and ScV-L-A. Each of them plays a different role. Genes present in ScV-M viral genome code for the production of a specific toxin (K1, K2 or K28). The stability and replication of ScV-M virus depends on the presence of the ScV-L-A virus (Wickner 1985; Magliani *et al.* 1997; Marquina *et al.* 2002). Non-Mendelian inheritance of the cytoplasmic viruses ensures that all progeny inherit it. Progeny gain the ability to kill sensitive individuals that do not carry the virus. It is important to stress that the killer yeast that produces the toxin is resistant to its own toxin. This makes it a different system than bacterial colicin

production. In the case of bacteria, the release of the toxic compound requires the lysis of the producer cell. Bacterial toxin production is thus considered either spite or, in case of clonal population, *indirect altruism* via kin selection (see glossary) (West *et al.* 2006).

Production of yeast viral-associated toxins is costly. In the case of a certain type of toxin (K1 toxin) it reduces fitness (measured as growth rate on the agar plate) by about 4% (Wloch-Salamon *et al.* 2008). The costly production of toxin pays off for killers mainly in structured environments, where they can kill resource-competing sensitive cells that are in close proximity. Then the killers can profit both from the nutrient 'saved', i.e. not used by killed cells and by scavenging nutrients released from the dead cells. All this allows killer populations to take over the sensitive cells, even in the case where the killer cells are initially rare (figure 3) (Wloch-Salamon *et al.* 2008). In such cases, killer phenomena could be classified as 'spite', when both actor's and recipient's fitness is reduced. However, loss of fitness is not the same for each of the players. Small reduction of killer growth rate (which population ultimately outgrows sensitive competitors) cannot be compared with much greater costs incurred by dead sensitive cells. Based on this it seems that toxin production is more an example of selfish behaviour, where killers win.

Yeast toxins are labile proteins that operate at a given temperature and pH. *S. cerevisiae* toxin K1 is not stable enough to exhibit any effects on a sensitive population in a non-structured condition (laboratory population mixed on agar plate or in liquid populations). In addition the killers' reduced fitness (measured as growth rate) caused by the presence of the viruses (compared to the fitness of the sensitives) causes the toxin-producing strains to decline in frequency in a mixed culture of killer and sensitive strains in a non-structured condition. It is interesting why in such conditions the presence of viruses is maintained in the yeast cells. Does the host yeast cell profit somehow, in a not yet known way, from the presence of the viral particles? How strong is the co-evolution of those two biological entities? There is a scarcity of research on these topics. Recent research shows that the presence of the viruses does not substantially change the transcriptome of the yeast cells, leading to the conclusion that the yeast–virus co-evolutionary bounds are strong (McBride *et al.* 2013). It might be that the whole story should be analysed from the perspective of the virus, 'promoting' self-replication within host cells. Then toxin production in the structured environment could be an example of the mutual benefit when both virus and killer host profit. In case of mixed environment, where only the virus profits (at least until so long as yeast cells persist in the population), it could be understood as virus selfishness.

4. Apoptosis – extreme altruism?

Programmed cell death (PCD) is an active (means that it need additional energy input) and genetically regulated type of cell death. Depending on the expressed distinct morphotypes it can be classified as apoptosis, paraptosis and autophagy (Galluzzi *et al.* 2012). Apoptosis is the most frequently reported type of death for yeast, so here I will concentrate on it. For a long time, the occurrence of apoptosis and even the possibility of its occurrence in unicellular organisms was thought to be theoretically unfounded (Sharon *et al.* 2009). This kind of death has been attributed only to complex organisms, in which the controlled processes of a single cell death could have an impact on the proper and efficient functioning of the whole organism. Currently, there is growing evidence that PCD is present in many unicellular organisms, such as protozoa, bacteria, slime moulds and yeasts (table 1). Yeast apoptotic cells are characterized by specific markers, many of them being similar to what is seen in higher multicellular organisms. This includes morphological features such as mitochondrial depolarization, reduction of cellular volume, chromatin condensation, nuclear fragmentation, loss of cell membrane integrity and plasma membrane blebbing (but maintenance of its integrity until the final stages of the process), as well as biochemical markers

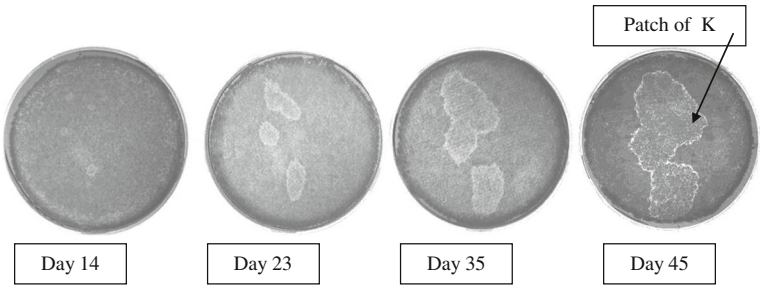

Figure 3. Invasion of killers in the originally mixed killer (K) and sensitive (S) populations. Stable environment (agar plates) allows increase of the K population in time. Populations of mixed K and S strains were transferred without changing their structure (using velvet cloth). We can observe patches of killers increasing in size during every transfer on the fresh medium allowing for regrowth of both K and S (photos: author) (photos of the 10 mL Petri dish, diameter ~6 cm).

Table 1. Convergent social phenomena in other microorganisms and *Saccharomyces cerevisiae*

Social phenomena	Microorganisms	Examples of the references (including reviews) applying to yeast
Domicile creation	Biofilms in many bacteria	Yeast biofilms (Reynolds and Fink 2001; Bojsen *et al.* 2012)
Specialised food provisioners	*Rhizobium* Cyanobacterial Heterocysts	Pseudohyphae (Gimeno *et al.* 1992; Cullen and Sprague 2012)
Specialized defenders	a) Colicin-producing *Escherichia coli*	a) Toxin producing yeast (Woods and Bevan 1968; Schmitt and Breinig 2006)
	b) *Myxobacteria* peripheral rods	b) Extracellular matrix (Kuthan *et al.* 2003)
		c) Drug efflux pump (Vachova *et al.* 2011)
Programmed cell death	*Escherichia coli,* protozoa, bacteria, slime moulds	Yeast apoptosis (Madeo *et al.* 1997; Honigberg 2011)
Communication via chemicals	a) Quorum sensing in bacteria,	a) Quorum sensing in yeast (Chen and Fink 2006)
	b) Pheromone signalling	b) Pheromone sensing in yeast (Bardwell 2004)
	c) Dimorphic switch	c) Formation of spores (Neiman 2011) or quiescent cells (Allen *et al.* 2004);

References applying organisms other that *S.cerevisiae* could be found for example in Crespi 2001 and West *et al.* 2006. Table adapted after Crespi 2001.

including phosphatidylserine (PS) exposure, mitochondrial membrane permeabilization and cytochrome c release, activation of proapoptotic yeast homologues of Bcl-2 family proteins (e.g. Ybh3p), and activation of caspases in the mutant of the cell cycle gene *CDC48* (Madeo *et al.* 1997; Wloch-Salamon and Bem 2013). At present there are 19 genes associated with yeast apoptosis (*http://www.yeastgenome.org/*). The most significant are *MCA1* (homologue to mammalian caspases), *AIF1* (apoptosis inducing factor) and *NUC1* (mitochondrial nuclease). Apoptosis has been observed during unsuccessful mating, meiosis and sporulation, long incubation in rain water, toxin exposure (including low concentrations of killer toxin), and has been associated with differentiation of cells within a colony, and with consecutive budding events (replicative aging) (Ivanovska and Hardwick 2005; Buttner *et al.* 2006).

Researchers in the field generally agree that apoptosis occurs in *Saccharomyces cerevisiae* (Sharon *et al.* 2009; Carmona-Gutierrez *et al.* 2010; Shemarova 2010; Wloch-Salamon and Bem 2013). However, evolution and maintenance of PCD processes in yeast and all single-celled organisms remains a particularly puzzling problem. It is difficult to explain why a self-contained organism would cause self-destruction (Nedelcu *et al.* 2011)? Can dying be a better strategy for an individual than living? When? And why? Who is the beneficiary of one's death? There have been attempts to explain the existence of the extreme behaviour of PCD in microorganisms by citing population-level benefits connected with scarce nutrient conditions, removal of weak, unhealthy, sterile, mutated or damaged cells, or protection of 'better' cells; thus facilitating population adaptation to new or changing environments (Buttner *et al.* 2006). However, in such an interpretation there is an implicit assumption of the presence of kin selection, where costs and benefits are estimated according to Hamilton fitness-based classification (see glossary). Only if the individual sacrifices itself for the sake of its relatives and their shared genes does it make evolutionary sense. For any altruistic behaviour to evolve *via* kin selection, it is not the average genetic similarity of the population that is important. Rather, what is important is the relatedness between an actor and a recipient compared to the relatedness between an actor and a random member of the population (Grafen 2006).

Consequently, the population-wide average of genetic similarity is meaningless in the absence of mechanisms or conditions that can promote 'nonrandom associations between genotypes (assortment)' (Hamilton 1971). These mechanisms could include: (1) kin recognition/discrimination, on which there is not much data except for the mentioned earlier flocculins (green beard gene) example; and/or (2) population genetic structure due to low rates or short ranges of dispersal, such that the interacting partners (i.e. those in close proximity) are more likely to be genealogically related (on account of population viscosity), which is usually the case in feral yeast growing on stable environments. However, there is lack of experimental or empirical data supporting either of these mechanisms, which is needed for final determination of its social meaning.

A recent comprehensive review highlights additional problems with some experiments on yeast apoptosis (Nedelcu *et al.* 2011). The deletion of the metacaspase gene (*YCA1*) prevents death under conditions that induce PCD (Madeo *et al.* 2002). Yeast metacaspase mutants Δ*yca1* has an advantage over wild-type strains, visible as increase in density at the initial stage of competition. However, ultimately, mutant Δ*yca1* lost in competition to the wild type. This was interpreted as PCD having a role at the population level in removing stress-

induced damaged or mutated cells. It was shown, however, that aged metacaspase mutants Δ*yca1* lost their ability to regrow on fresh medium and accumulated more mutations (Vachova and Palkova 2005) and have a higher content of detrimental oxidized proteins (Jamieson 1995) than the wild type, even in the absence of stress (Sigler *et al.* 1999; Khan *et al.* 2005). Yet, the inactivation of the metacaspase gene has a negative effect on individual fitness. A similar example is provided by the glutaredoxin 2 gene (*GRX2*) (Gomes *et al.* 2008). Those two examples confirm the ubiquity of gene pleiotropy which cannot be neglected (Stearns 2010). Blocking of PCD in experiments aimed at addressing its benefits should be performed in ways that are not likely to interfere with other cellular activities that may have a non-PCD-related effect on cell fitness (Nedelcu *et al.* 2011). An alternative explanation for the existence of apoptosis in yeast is the suggestion that PCD might be an unavoidable outcome of the detrimental metabolic imbalances (e.g. Bidle and Falkowski 2004; Nedelcu *et al.* 2011)

In actively growing yeast cells, when growth is arrested by some form of sub-lethal stress, energy utilization becomes uncoupled from energy production, and this can lead to an oxidative burst, resulting in cell death (Eisenberg *et al.* 2007). This scenario is consistent with the observation that under the same PCD-inducing conditions, cells from exponentially grown cultures (or nonquiescent cells) are more likely to undergo PCD compared to cells from stationary phase (or quiescent cells). For instance, in aging yeast cultures, nonquiescent cells (i.e. those that continue to divide after the exhaustion of glucose in the medium) are much more likely to develop apoptotic markers than the quiescent/resting cells (figure 4) (Allen *et al.* 2006). According to this scenario, PCD is beneficial for the young cells where effective energy production allows for faster growth, but becomes an increasing problem with resource depletion The occasional expression of PCD is triggered by metabolic imbalances between the cytosolic and mitochondrial compartments that would trigger the overproduction of reactive oxygen species (ROS) (Blackstone and Green 1999). Consistent with this scenario is the fact that the mitochondrion is the central executioner in apoptosis (Eisenberg *et al.* 2007; Galluzzi *et al.* 2012), and the fact that most environmental types of stress that induce PCD also result in the overproduction of ROS (Carmona-Gutierrez *et al.* 2010). Nevertheless, the seemingly maladaptive trait of PCD could – under conditions in which kin selection or group selection can act – be co-opted as an altruistic trait. How can this occur? If social group-living signals (either chemical or position-dependent signals) can simulate the ancestral PCD-inducing signal (e.g. ROS; figure 3), and if this group-induced signal-dependent death is beneficial (at the group level), such types of PCD might be selected for and evolve into altruistic adaptations. This might be the case of the evolved numerous clonal aggregates where the older cells, located inside the group, show markers of apoptotic death (Ratcliff *et al.* 2012). Well-planned experiments could explain if such altruism allows for better spread of the genes of clonal populations.

5. Perspectives

Expanding numbers of microbial sociobiology research papers (Crespi 2001; Rainey and Rainey 2003; Griffin *et al.* 2004; Nadell *et al.* 2008; Ross-Gillespie *et al.* 2009; Xavier *et al.* 2009; Mitri *et al.* 2011; Nanjundiah and Sathe 2011; Xavier *et al.* 2011) have proved the importance of adding yeast to other sociobiological model systems (Aerts *et al.* 2011; Greig and Travisano 2004; West *et al.* 2006; Foster *et al.* 2007; McBride *et al.* 2008; Smukalla *et al.* 2008; MacLean *et al.*

Table 2. Examples of the yeast behaviour classified as social

Social phenomena	Classification	References
Flocculation	Cooperation, 'green beard gene'	Smukalla *et al.* 2008; Veelders *et al.* 2010; Bruckner and Mosch 2012
Invertase production	Cooperation; 'public good' production;	Greig and Travisano 2004; Gore *et al.* 2009; Koschwanez *et al.* 2011
Toxin production	a) Spite (interference competition)	a) Wloch-Salamon *et al.* 2008
	b) Viral-yeast mutualism	b) McBride *et al.* 2008, 2013
Colony and biofilm formation	Cooperation; 'public good' production	Honigberg 2011; Vachova *et al.* 2011; Cap *et al.* 2012; Vachova *et al.* 2012
Apoptosis	Altruism (social meaning requires confirmation)	Madeo *et al.* 2002; Buttner *et al.* 2006; Gomes *et al.* 2008
Dimorphic shift	Cooperation (chemical signal)	a) Ohkuni *et al.* 1998; Piccirillo and Honigberg 2010
a) Spore formation		b) Allen *et al.* 2006; Aragon *et al.* 2008; Davidson *et al.* 2011
b) Quiescent cell formation		

2010; Koschwanez *et al.* 2011) (table 1). This also reflects the increased interest of the scientific community in this topic. There is an agreement among scientists about the sociobiological meaning of flocculation, invertase secretion and killing ability (table 2). Still, the social importance of programmed cell death needs some further confirmation and clarification (Nedelcu *et al.* 2011) (table 2). There is need for further well-planned experiments that will allow for confirmation of the social meaning of observed traits while controlling pleiotropic effects of genes. In my opinion, Hamilton's conceptual framework together with the recent findings in all branches of science provides a great base for addressing these questions.

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Glossary

Apoptosis	A type of programmed cell death characterized by specific morphological and biochemical features. In a multicellular context, the term 'programmed' has been used to imply two different issues. The first implication is that some cells are destined to die. This is not relevant to unicellular microorganisms. Second, 'programmed' implies that cells die following an internal, genetically encoded death program that ensures an organized death in response to either stress or developmental factors (Nedelcu *et al.* 2011)
Biofilms	Microbial biofilms are populations of microorganisms that are concentrated at an interface (usually solid–liquid) and typically surrounded by an extracellular polymeric substance (EPS) matrix. Aggregates of cells not attached to a surface are sometimes termed 'flocs' and have many of the same characteristics as biofilms (Hall-Stoodley *et al.* 2004)
Direct fitness	The component of fitness gained through reproduction (West *et al.* 2006)
Indirect fitness	The component of fitness gained from aiding the reproduction of non-descendant relatives (West *et al.* 2006)
'Green beard' genes	Genes that code for a conspicuous phenotype that can be used to discriminate between carriers and non-carriers of the gene, and that induce a carrier to behave altruistically toward another carrier, irrespective of the genetic relatedness at other loci between the two partners. This mechanism emphasizes that, in terms of relatedness, what is most important for altruism to evolve is genetic relatedness at the locus providing altruistic behaviour (i.e. the probability that interacting partners have the same allele) as opposed to genealogical relationship over the entire genome (Gardner and West 2010)
Hamilton's rule	A condition ($rb-c > 0$) that predicts when a trait is favoured by kin selection, where c is the cost to the actor for performing the behaviour, b is the benefit to the individual whom the behaviour is directed towards, and r is the genetic relatedness between those individuals (West *et al.* 2006)
Indirect altruism	A behaviour that increases the frequency of another individual's genes at a cost to one's own fitness
Kin selection	A process by which traits are favoured because of their beneficial effects on the fitness of relatives (West *et al.* 2006)
Pseudohyphae	Chains of elongated (i.e. filamentous) budding diploid cells (Honigberg 2011)
Public goods	A resource that is costly to produce, and provides benefit to all the individuals in the local group or population (West *et al.* 2006)
Quorum sensing	The ability to respond to local population density. For microorganisms, this occurs by the secretion of self-produced quorum-sensing molecules (autoinducers). The concentration of these molecules is an indicator of local population density, and increasing the concentration of a QS molecule to a threshold level induces a population-wide phenotypic change (Honigberg 2011)
Yeast aggregates	Groups of yeast cells resulting from incomplete separation of 'daughter cells'
Yeast flocks	Yeast flocculation is regulated by adhesin proteins ('flocculins'). Flocculating cells also produce a mixture of polysaccharides around the exterior of the cell, called the extracellular matrix (ECM)

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