# Selection against somatic parasitism can maintain allorecognition in fungi

Tamas Czárán [a], Rolf F. Hoekstra [b], Duur K. Aanen [b,*]

[a] MTA-ELTE Research Group of Theoretical Biology and Evolutionary Ecology, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary
[b] Laboratory of Genetics, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

## ABSTRACT

Fusion between multicellular individuals is possible in many organisms with modular, indeterminate growth, such as marine invertebrates and fungi. Although fusion may provide various benefits, fusion usually is restricted to close relatives by allorecognition, also called heterokaryon or somatic incompatibility in fungi. A possible selective explanation for allorecognition is protection against somatic parasites. Such mutants contribute less to colony functions but more to reproduction. However, previous models testing this idea have failed to explain the high diversity of allorecognition alleles in nature. These models did not, however, consider the possible role of spatial structure. We model the joint evolution of allorecognition and somatic parasitism in a multicellular organism resembling an asexual ascomycete fungus in a spatially explicit simulation. In a 1000-by-1000 grid, neighbouring individuals can fuse, but only if they have the same allotype. Fusion with a parasitic individual decreases the total reproductive output of the fused individuals, but the parasite compensates for this individual-level fitness reduction by a disproportional share of the offspring. Allorecognition prevents the invasion of somatic parasites, and *vice versa*, mutation towards somatic parasitism provides the selective conditions for extensive allorecognition diversity. On the one hand, if allorecognition diversity did not build up fast enough, somatic parasites went to fixation; conversely, once parasites had gone to fixation no allorecognition diversity built up. On the other hand, the mere threat of parasitism could select for high allorecognition diversity, preventing invasion of somatic parasites. Moderate population viscosity combined with weak global dispersal was optimal for the joint evolution of allorecognition and protection against parasitism. Our results are consistent with the widespread occurrence of allorecognition in fungi and the low degree of somatic parasitism. We discuss the implications of our results for allorecognition in other organism groups.

## 1. Introduction

Cooperation is predicted to evolve more easily if social interactions predominantly occur between genetically related individuals (Bijma and Wade, 2008; Hamilton, 1964; West et al., 2007). Positive assortment between related individuals can be achieved by high population viscosity or by kin discrimination, which can either be based on shared environment or on genetic cues (Grafen, 1990). Genetic, cue-dependent kin recognition is common in all domains of life, including plants (Chen et al., 2012; Dudley and File, 2007), fungi (Aanen et al., 2008; Glass and Dementhon, 2006; Saupe et al., 2000), bacteria (Gibbs et al., 2008), vertebrates (Charpentier et al., 2007), insects (van Zweden and d'Ettorre, 2010),

slime moulds (Hirose et al., 2011; Strassmann et al., 2011) and sessile marine invertebrates (Grosberg, 1988). However, the origin and maintenance of polymorphic genetic recognition cues remain incompletely understood despite substantial theoretical and empirical research (e.g. (Crozier, 1986; Nauta and Hoekstra, 1994; Rousset and Roze, 2007)). In this paper, we address the evolution of a specific example of kin recognition, allorecognition in multicellular (filamentous) fungi.

A multicellular individual essentially is a colony of cells, which cooperate to increase their inclusive fitness, for example by division of labour or by size-related protection against predation (Buss, 1987; Gavrilets, 2010; Ispolatov et al., 2012; Koschwanez et al., 2011). Extant multicellular organisms represent different stages in the transition towards individuality (Queller and Strassmann, 2009). In the most derived forms, the multicellular individual has become the new unit of selection, as adaptations at this level, such as an early germline–soma differentiation, render

* Corresponding author.
    *E-mail addresses:* czaran@caesar.elte.hu (T. Czárán), hoekstra.rf@gmail.com (R.F. Hoekstra), duur.aanen@wur.nl (D.K. Aanen).

somatic cells evolutionarily dead ends (Bourke, 2011; Buss, 1987; Frank, 2003; Okasha, 2006). However, organisms with indeterminate growth, such as fungi, do not have an early germ–soma differentiation, so that all body parts retain the potential to reproduce and therefore still are "hopeful reproductives" (Aanen et al., 2008). Fungi further differ from other multicellular organisms in their growth mode. They form filaments (hyphae) that are branching and fusing regularly to form a dense, radially growing, network called the mycelium. Each fragment can reproduce via fission or the formation of asexual spores. In contrast to most other multicellular organisms, cell compartmentalization is not very strong and in some fungi nuclei can freely move through parts of the mycelium (Roper et al., 2011). Therefore, the cooperating units in the fungal colony are the haploid nuclei (Rayner, 1991). Colony size can be increased through hyphal fusion between germinating spores during colony establishment and between the hyphae of mature colonies (Read et al., 2010; Roca et al., 2005). Fusion between mycelia can be mutually beneficial (Aanen et al., 2009; Bastiaans et al., submitted for publication; Pontecorvo, 1958; Richard et al., 2012).

In spite of the potential benefits of fusion between individuals, fusion between different mycelia is restricted by genetic allorecognition systems based on gene polymorphisms at several loci, restricting fusion almost exclusively to clonally related colonies (Aanen, 2010; Glass et al., 2000; Saupe, 2000; Saupe et al., 2000). Successful fusion between colonies requires matching at all recognition loci otherwise mycelia are somatically incompatible and fusion will be interrupted by programmed cell death of the fused hyphal compartments at the border of two colonies. The widespread occurrence of allorecognition suggests that the disadvantages of fusion on average will be greater than the benefits (Nauta and Hoekstra, 1994). The most generally accepted hypothesis is that allorecognition has evolved to limit the opportunities for somatic parasites (e.g. (Aanen et al., 2008; Buss, 1982, 1987; Buss and Green, 1985; Grafen, 1990; Grosberg and Strathmann, 2007; Nauta and Hoekstra, 1994; Rousset and Roze, 2007). A somatic parasite is a variant that contributes less to colony functions, but relatively more to reproduction. Within a colony of cooperating nuclei, such a variant will be selected, but selection among colonies will disfavour such a variant. Thus, it is a cheater as it increases its relative fitness within a colony of wildtype nuclei, but does so at the cost of colony fitness (Ghoul et al., 2014). Although such mutants are not common in fungi, a few examples are known (Davis, 1960; Pittenger and Brawner, 1961).

Although it makes intuitive sense that allorecognition has evolved as a protection against somatic parasitism, its evolution is not well understood. First, Crozier (1986) pointed out that short-term selection will work against the genetic diversity of cues required for allorecognition. If fusion provides a benefit, or if rejection is costly, the common allele will always be favored, because it will fuse more often than rare alleles. Therefore, allorecognition 'eats up' the genetic variation upon which it crucially relies, a prediction now known as 'Crozier's paradox' (Aanen et al., 2008; Crozier, 1986; Rousset and Roze, 2007). The balance between short-term positive frequency-dependent selection limiting allorecognition diversity, as predicted by Crozier, and the long-term risk to be hit by a somatic parasite (or a 'cheat'; (Ghoul et al., 2014; Grafen, 1990), selecting for increased allorecognition diversity, thus remains unknown. Second, even under the assumption of potentially negative fitness consequences of somatic fusion, theoretical modelling predicts only limited polymorphism for allorecognition, and cannot explain the extreme extent to which polymorphism can go in many cases (Grosberg and Quinn, 1989; Jansen and van Baalen, 2006; Nauta and Hoekstra, 1994). Although the Nauta and Hoekstra model could explain the maintenance of a limited number of allotypes once they were already above a certain

threshold frequency in the population, it could not explain the invasion of new allotypes starting from very low frequencies. However, this model did not take spatial structure into account.

In the present study, we test the hypothesis that the potential for somatic parasitism can select for allorecognition, and *vice versa*, that allorecognition keeps somatic parasites at a low frequency, using a spatially explicit model. We model the joint evolution of allorecognition and somatic parasitism in an asexual multicellular ascomycete fungus with the potential for somatic fusion. In our model, initially no parasitism and no allorecognition exist, *i.e.* every individual can fuse with every other. Mutation at the parasite locus can generate a nuclear parasite, while mutation at the allorecognition locus can generate new allorecognition types (allotypes). Somatic fusion is only possible between individuals with identical allotypes. The parasitic allele may spread horizontally by somatic fusion in compatible inter-individual confrontations. We systematically assess the effect of different assumptions about the details of parasitism and about the fitness consequences of fusion, and especially about the effect of spatial structure on both the allorecognition diversity and the level of parasitism evolving. Our study shows that allorecognition contributes to the stability of multicellular growth by preventing the invasion of somatic parasites, and *vice versa*, that the potential for somatic parasitism can select for extensive allorecognition diversity, thus solving Crozier's paradox.

## 2. Methods

### 2.1. The model

The model is a spatially explicit cellular automaton (CA) with which we addressed the joint evolution of allorecognition and somatic parasitism in a modular, sedentary multicellular organism, which produces propagules, such as spores (Fig. 1). However – since application to other biological systems in which somatic fusion occurs is straightforward – we will use a more general terminology in the text throughout.

The basic assumptions of the model are the following:

1. Multicellular individuals are sedentary and occupy a 2D habitat represented by a $1000 \times 1000$ square lattice of toroidal topology. Each site of the lattice harbours one multicellular individual.

2. The organisms are haploid and reproduction is exclusively asexual through mitotic propagule formation. Therefore, we can simplify the genetic specification of the allorecognition system by assuming a single locus with a maximum of 50 different alleles. Thus the population contains 50 different allorecognition types or *allotypes* at most.

3. The individuals are identical in all but two respects: they may carry different alleles at the allorecognition locus, and either a parasitic ($h$) or a non-parasitic ($H$) allele at a "Parasitism" locus (which can be a functionally connected set of loci, of course). Every allorecognition allele can mutate with small probability ($10^{-6}$ per generation) into any other one of the 49. Also, a non-parasitic $H$ individual may produce a mutant parasitic $h$ offspring with probability $10^{-6}$ per generation; no back mutation from parasites to non-parasites is allowed. We have also tested a tenfold higher mutation rate towards parasitism ($10^{-5}$ per generation).

4. Neighbouring individuals can fuse if they have the same allorecognition allele. Such fusions may result in extended chimaeric individuals that occupy more than one patch, but the actual effects of fusion remain local – each individual component in the chimaera feels the effect of fusion with just its four immediate neighbours (*i.e.*, within its Neumann neighbourhood). For extended individuals that occupy more than one patch, all calculated fitness