FUNGAL EVOLUTION

Old fungus, new trick

A secreted effector from the plant pathogenic fungus *Ustilago maydis* has evolved to acquire a new function that contributes to the unique lifestyle of this species, highlighting the utility of using comparative genetic analyses to address current questions in plant–microorganism interactions.

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Smut fungi are a diverse and ancient group of plant pathogens, comprised of nearly 1,500 species predicted to have emerged more than 250 million years ago (mya)[1]. This group of fungi are characterized by a biotrophic lifestyle — requiring living plant tissue to reproduce — and often spread systemically before causing disease symptoms in reproductive organs[2]. Smut species infect various grass hosts, including maize, wheat, barley and sugar cane[2]. Two smut fungi, *Ustilago maydis* and *Sporisorium reilianum*, have convergently evolved to infect maize following its domestication ~10,000 years ago, despite these two species having diverged around 20 mya (Fig. 1)[3]. *S. reilianum* has a pathogenic lifestyle typical of smut fungi, with its symptoms restricted to inflorescences, whereas *U. maydis* can cause symptoms in all maize vegetative tissue[4]. In their study, recently published in *Nature Microbiology*, Tanaka et al.[3] compare these two smut fungi to uncover one of the molecular determinants underpinning the unique pathogenic lifestyle of *U. maydis*.

Plant pathogens secrete effectors — proteins and small molecules that alter plant physiology by targeting host processes — to aid infection and colonization. Tanaka et al.[3] show that a secreted effector, Tin2, common to a number of smut fungi, has acquired a new function in *U. maydis*; an evolutionary process known as neofunctionalization. They discovered that the *U. maydis* and *S. reilianum* Tin2 effector orthologues target different paralogues of a maize kinase during infection, leading to their stabilization and inhibition, respectively (Fig. 1)[3]. The two Tin2 effectors displayed different activities, with only the *U. maydis* Tin2 inducing host production of a red pigment called anthocyanin[3]. A resurrected ancestral Tin2 could complement the virulence function of the *S. reilianum* Tin2, but did not substitute the virulence function of the *U. maydis* Tin2 (ref. [3]). The authors hypothesize that the neofunctionalization of the *U. maydis* Tin2 may be related to the unique ability of this fungus to induce leaf tumours,

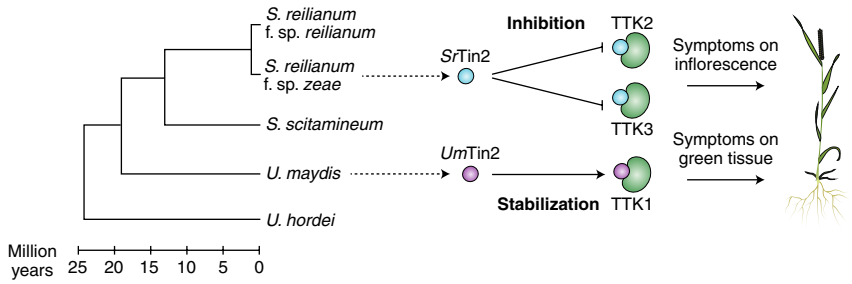

Fig. 1 | *U. maydis* and *S. reilianum* Tin2 effector orthologues target different paralogues of maize kinases. Left, Phylogenetic tree and divergence estimation of five representative smut fungi species, *S. reilianum* f. sp. *reilianum* (sorghum host), *S. reilianum* f. sp. *zeae* (maize host), *Sporisorium scitamineum* (sugar cane host), *U. maydis* (maize host) and *Ustilago hordei* (barley host). **Middle**, The mechanism by which Tin2 orthologues function in maize. The *S. reilianum* Tin2 (*Sr*Tin2) inhibits the maize host kinases TTK2 and TTK3, while the *U. maydis* Tin2 (*Um*Tin2) stabilizes the paralagous maize kinase, TTK1. **Right**, Disease symptoms caused by *U. maydis* and *S. reilianum* fungi. Left panel adapted from ref. [7], Oxford Univ. Press.

possibly by diverting host resources towards anthocyanin production and away from lignin synthesis, which would otherwise restrict fungal proliferation[5].

Filamentous plant pathogens, such as fungi and oomycetes, deliver two major classes of effectors to distinct sites in the host plant[2,6]. Whereas apoplastic effectors are secreted into the plant extracellular space, cytoplasmic effectors, such as Tin2, are translocated inside the plant cell. Both classes of effectors can activate host immune receptors encoded by disease resistance (*R*-) genes and trigger a robust immune response that blocks pathogen infection. One of the peculiarities of the *U. maydis* pathosystem is that there are no known *R*- genes against this pathogen, either in maize or the ancestral host, teosinte. This is remarkable considering that a number of effectors, such as Tin2, have evolutionary histories that date back to at least 20 mya. A lack of selection pressure imposed by host immune receptors may explain why Tin2 is not among the effectors recently identified to be under positive selection between *U. maydis* and *S. reilianum*[7]. Thus, both the evolutionary dynamics and persistent nature of Tin2 contrast with fungal effectors that have an avirulence activity, notably, some of the effectors of the rice blast fungus *Magnaporthe oryzae*[8].

The mechanism by which filamentous plant pathogens translocate effectors into host cells remains an unsolved mystery[9]. The cytoplasmic effectors of oomycetes carry a distinct domain between the signal peptide and the C-terminal 'effector domain' that is essential for translocation[10]. In contrast, there are no apparent domains in fungal effectors that are required for delivery into plant cells. The *U. maydis* experimental system should be particularly amenable for studies to define the genetic determinants of host translocation in cytoplasmic effectors, such as Tin2. Mutants of Tin2 that retain the capacity to bind host kinases, but fail to genetically complement *U. maydis*, may reveal the amino acid residues necessary for host translocation.

The paper by Tanaka et al.[3] is an elegant example of how genetic diversity can be used to study the evolution of pathogen effectors. The authors took the uncommon approach of reconstructing the predicted ancestral sequence of Tin2 to experimentally challenge the hypothetical models of its

evolutionary history. In this, Tanaka et al.[3] determined that the function of the *U. maydis* Tin2 is evolutionarily derived and appears to contribute to the pathogenic lifestyle of this species (Fig. 1). Ancestral sequence reconstruction has emerged as an important approach to characterize extinct proteins[11], but has rarely been applied in the field of plant–microorganism interactions. Both this study[3] and a previous publication[12] have shown that experimental analyses of an ancestral effector can transcend phylogenetic inference to yield more accurate evolutionary models.

Tanaka et al.[3] provide yet another example of the value of *U. maydis* as an experimental system for molecular plant pathology. There are countless open questions about effector biology that this pathosystem can help to address. What are the genetic determinants of host specificity? What are the biochemical signals that trigger pathogen disease progression — are they host-derived, or are they part of an intrinsic developmental program? What are the genetic source materials for novel effector proteins, and what are the evolutionary strategies for generating such genomic dynamism? Also, how are effectors translocated inside host cells? Undoubtedly, further comparative genetic analyses will reveal more of the tricks, both recent and ancient, that the old smut fungi have at their disposal. ❐

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Competing interests

The authors declare no competing interests.