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Oomycete–plant coevolution: recent advances and future prospects

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Oomycetes are a diverse group of eukaryotic organisms that have colonised many ecological niches; yet more than 60% of the known species are parasitic on plants. Parasitism of plants has evolved several times independently in three different lineages of the Oomycota. Here, we provide an overview of the current knowledge of the diversity, evolution and lifestyles of plant parasitic oomycetes. We then report on recent advances in molecular studies on oomycete–plant interactions with a particular emphasis on work with oomycete effectors. In the future, genome sequencing of a broader spectrum of oomycete species will expand our knowledge of pathogenicity mechanisms and will likely reveal novel structural and functional classes of effectors.

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Introduction to the oomycetes

Oomycetes are a diverse group of eukaryotic organisms with a worldwide distribution. They can be found in terrestrial mountains as well as in open sea environments. They have not only colonised the deserts of Iran [1], but also the arctic regions of the world, including Antarctica [2,3]. Nonetheless, the oomycetes remain relatively poorly known compared to the true fungi, the Mycota, to which they are unrelated phylogenetically.

A common feature of all oomycetes is their ability to directly absorb nutrients (osmotrophic lifestyle), which is the main reason they have long been confused with the fungi, with which they share several convergent traits such as hyphal growth and dispersal by mitotically formed spores. However, the tinsel flagellum with tripartite hairs on the biflagellate motile stages of the oomycetes revealed their true phylogenetic affinity with diatoms and brown algae in the kingdom Straminipila [4,5]. Some oomycetes are parasites that develop within a single host cell, for example a diatom, with the entire cytoplasm eventually recruited to reproduction (holocarpy) [6]. Other oomycetes can form dense mats of long, branched hyphae, like in *Leptomitus lacteus*, the sewage oomycete [7]. Several oomycete groups have evolved into highly adapted pathogens affecting organisms in eukaryotic kingdoms as diverse as Alveolata, Animalia, Mycota, Straminipila and Plantae. The majority of oomycete species are parasites of plants, and some species, such as the potato late blight agent *Phytophthora infestans*, cause diseases of great importance to mankind. Unsurprisingly, most of the research on oomycetes focuses on plant pathogenic species [8•].

Oomycete evolutionary history: current status

Oomycetes have most likely evolved in a marine environment, as the most basal lineages of the Oomycota are predominantly marine parasites of seaweeds, diatoms, crustaceans and nematodes (Figure 1). Despite their great genetic divergence, these basal lineages account for only about 5% of oomycete species, whereas more than 60% of the species are plant parasitic. Parasitism of plants has evolved at least three times independently in different lineages of the Oomycota (Figure 1). It emerged once in the Saprolegniales in which the legume pathogen *Aphanomyces euteiches* is becoming an important subject of study [9–11], and at least twice in the peronosporalean lineage, which includes widely known plant pathogens, such as downy mildews, *Phytophthora*, *Pythium* and *Albugo*. In addition, evolution from a presumably saprophytic ancestor into obligate biotrophic pathogens has occurred twice in the peronosporelean lineage, leading to white blister rusts (Albuginales) and downy mildews (part of the Peronosporaceae) (Figure 1).

The Albuginales are an ancient lineage with several traits that underscore their intimate adaptation to plant pathogenicity. Such traits include the lack of necrotrophy even in severely infested plant tissue (Figure 2i), and independence from atmospheric water for sporulation, a unique oomycete trait that is determined by highly regulated, enzyme mediated sporulation and pustule opening mechanisms [12]. Apparently, the host immune system is strongly manipulated by white blister rusts resulting in increased susceptibility towards other pathogens [13]. In addition, contrary to earlier assumptions, most species of the Albuginaceae were recently shown to be highly host specific [14,15], and a specialised species,

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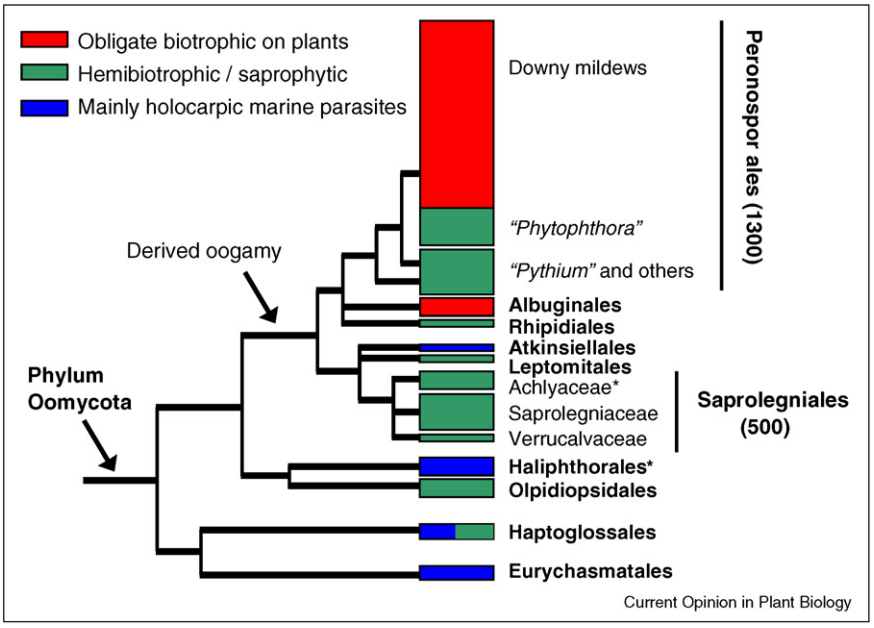
Figure 1



Schematic overview of the current knowledge on oomycete evolutionary history. Names in inverted commas denote taxa in need of taxonomic revision. Taxa that are not yet formally introduced are marked with an asterisk. Species numbers are indicated for the two largest oomycete orders.

Albugo laibachii, was recently found to occur on the model plant *Arabidopsis thaliana* [16••]. *Albugo candida* is the only white blister rust species known so far with a broad host range including three related families, the Brassicaceae, Capparaceae, and Cleomaceae [17]. The basis of this broad host range is enigmatic, but is possibly mediated by yet to be identified effectors that interfere with immune pathways that are conserved among these related host families.

In the Peronosporales, biotrophy has evolved gradually, from opportunistic pathogens among *Pythium*-like ancestors, to hemibiotrophy in *Phytophthora* species, which, unlike the obligate biotrophs, are also able to grow on dead host tissue (necrotrophy). Some Peronosporaceae evolved a high degree of host specialisation and a prolonged biotrophic phase of infection, ultimately leading to obligate biotrophy with decreasing impact on host fitness (Figure 2a–f). Evolution towards obligate biotrophy was most likely accompanied by the remarkable expansion and diversification in effector proteins, such as the host-translocated RXLR effectors, which have not been identified outside the Peronosporales [18–20]. Genetic isolation of the obligate biotrophs driven by host specialisation is pronounced. This has led to extensive radiation and speciation in several downy mildew genera, especially in *Hyaloperonospora*, *Peronospora* and *Plasmopara*, resulting in a total of about 800 downy mildew species described to date. Recently, the basal graminicolous downy mildew lineages were shown to have intermediate characteristics between downy mildews and *Phytophthora* suggesting step-by-step evolution from necrotrophy to obligate biotrophy [21].

Diversity of extended phenotypes caused by oomycetes on host plants

Several plant pathogens alter the morphology of their hosts in ways that improve colonisation, survival and dispersal. Such extended phenotypes suggest that host manipulation by pathogen effectors is more complex than the alteration of host immunity [22]. Among the most dramatic extended phenotypes of oomycete pathogens are the witches broom phenotypes caused by several graminicolous downy mildews (Figure 2g) [23,24]. In these cases, systemic infection by the oomycete leads to the replacement of flowers by shoots, which are often heavily infected and will produce massive amounts of both asexually formed sporangia and sexually formed oospores. Witches brooms provide the pathogen the advantage of being exposed to wind and rain, ensuring more efficient distribution of spores. This strategy has been further optimised in the floricolous downy mildews, which are members of a monophyletic clade within the genus *Peronospora* [25]. The floricolous downy mildews parasitise Asteranae *sensu* Chase & Reveal [26], and exclusively sporulate on floral parts of the plants thereby exploiting pollinating insects for dispersal. Many downy mildews and white blister rusts can infect the generative organs of their hosts (Figure 2h–k) and disperse with

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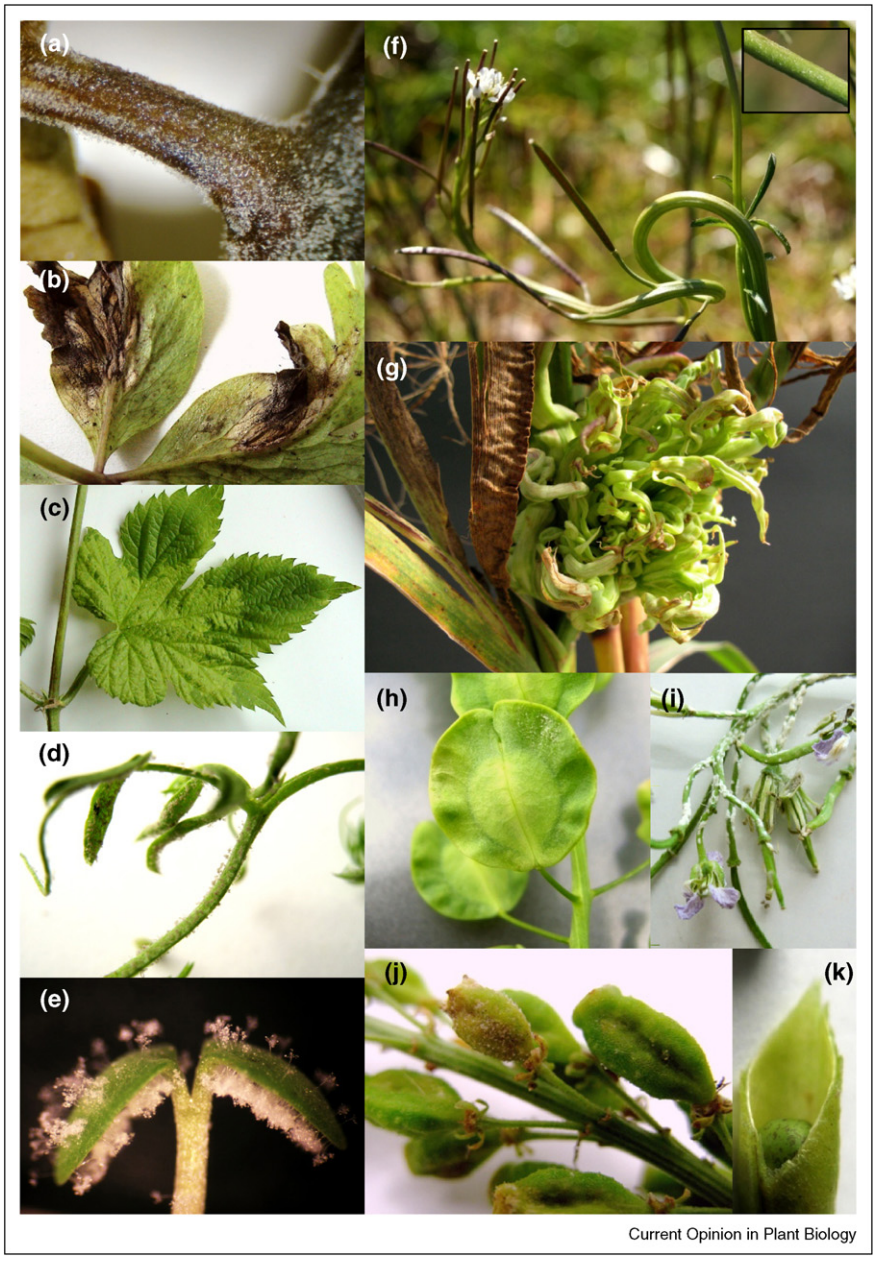
Figure 2



Diversity of symptoms caused by oomycetes on their host plants. **(a)** Necrotrophic growth of *Phytophthora infestans* on tomato. **(b)** Necrosis of *Anemone nemorosa* after sporulation of *Plasmoverna pygmaea*. **(c)** Chlorotic lesion caused by the systemic growth of *Pseudoperonospora humuli* in hop. **(d)** Systemic colonisation and sporulation of *Peronospora* sp. on *Vicia tetrasperma*, which hardly alters the host appearance except for slight chlorosis. **(e)** Profuse sporulation of *Hyaloperonospora thlaspeos-perfoliati* on its host (*Microthlaspi perfoliatum*) without additional visible symptoms. **(f)** Distortion of *Cardamine hirsuta* caused by *Hyaloperonospora* sp., with only limited sporulation and no adverse effect on inflorescence development. **(g)** Witches broom of *Pennisetum glaucum* caused by *Sclerospora graminicola* (image courtesy of Sabine Telle). **(h)** Limited sporulation of *Hyaloperonospora thlaspeos-arvensis* on seed pods of *Thlaspi arvense*. **(i)** Intense infection of *Cardamine pratensis* by *Albugo* sp., with sporulation, but without necrosis. **(j)** Systemic infection and sporulation of *Hyaloperonospora crispula* on a seed pod of *Reseda lutea*. **(k)** Opened seed capsule of *Vicia tetrasperma* systemically infected by *Peronospora* sp., with nearly normal seed development.

seeds [27–30]. Some studies have demonstrated the presence of oomycetes within the seeds of their hosts, which most likely enables pathogen survival and dispersal [31–33] (Heller and Thines, unpublished results).

Recent advances in the study of oomycete–plant interactions

Like other plant pathogens, oomycetes manipulate their hosts by secreting an arsenal of proteins, collectively

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known as effectors, which target plant molecules and alter plant processes [22,34,35]. Here, we highlight some recent advances in the study of oomycete–plant interactions with an emphasis on effector biology

Oomycete genomics: effectors populate dynamic and expanded genome regions

The genomes of several oomycetes have been sequenced but so far only those of *P. sojae*, *P. ramorum* and *P. infestans* have been described in publications [18,20]. The genome of *P. infestans* is remarkable by its large size. At 240 Mbp it is three to fourfold larger than the genomes of *P. sojae* and *P. ramorum* [18]. This expansion was driven by a dramatic increase in mobile elements and other repetitive DNA which account for ~74% of the *P. infestans* genome [18]. Most gene families are not expanded. However, the RXLR and CRN families of effectors are expanded twofold or more in *P. infestans* relative to *P. sojae* and *P. ramorum* [18]. These effector genes occupy regions of the genome that are rich in repeats and poor in genes [18]. Haas *et al.* [18] proposed that these genome regions are dynamic and accelerate effector evolution, possibly by enabling nonallelic homologous recombination and tandem gene duplication. Genome sequences of species that cover the broader phylogenetic spectrum of oomycete pathogens promise additional insights. Already, analyses of the transcriptomes of *Saprolegnia parasitica* [36], *Aphanomyces euteiches* [9] and *Pythium ultimum* [37] revealed distinct repertoires of effectors and pathogenicity determinants, highlighting the value of comparative genomics.

Copy number variation in effector genes impacts pathogen fitness

Segmental duplication of near-identical copies and copy number variation (CNV) are frequent in RXLR effector genes of *P. sojae* and *P. ramorum* [38••]. A significant number of predicted RXLR effector genes, ranging from 17% in *P. sojae* to 65% in *P. ramorum*, were estimated to be present in two or more copies [38••]. Three *P. sojae* effector genes, *Avr1a*, *Avr3a* and *Avr3c*, which confer avirulence to their cognate soybean *Rps* resistance genes, occur in tandem repeats and display CNV [38••,39]. CNV impacts transcript accumulation of *Avr1a* and *Avr3a* and virulence on resistant soybean plants highlighting an unusual mechanism of genetic adaptation to host resistance [38••]. Another intriguing case of CNV involves the *P. infestans Avr3b-Avr10-Avr11* locus, which determines avirulence to three potato resistance genes *R3b*, *R10* and *R11* [40]. A candidate avirulence gene, *pi3.4*, was identified in this locus and found to encode a large protein with the features of a transcriptional regulator [40]. In avirulent strains, the *Avr3b-Avr10-Avr11* locus displays copy number variation resulting in amplification of up to 25 truncated copies of *pi3.4* [40]. This case further emphasizes the occurrence of CNV in *Phytophthora* genes associated with virulence/avirulence. However, the mechanisms that enable these genome-level structural polymorphisms to impact phenotypic variation remain to be determined.

Effector trafficking into plant cells: How?

Oomycete RXLR effectors carry an N-terminal domain that enables translocation inside host cells and is similar in sequence, position and function to the PEXEL host translocation signal of malaria parasites [41–45]. How the RXLR domain enables translocation inside host cells is an important and challenging question. Dou *et al.* [46] reported that RXLR effectors of *P. sojae* enter plant cells in the absence of the pathogen. They showed that the RXLR motif of *P. sojae* Avr1b effector is required for cell death induction when a full-length construct with the signal peptide is expressed in soybean cells, presumably to enable re-entry of the protein following secretion [46]. In addition, Dou *et al.* [46] demonstrated that recombinant fusion proteins of the RXLR domain and the green fluorescent protein directly penetrate soybean root cells. These experiments suggest that RXLR effectors do not require a pathogen-derived machinery to traffic inside host cells and exclusively exploit host-derived molecules. Surprisingly, these results markedly differ from the prevailing model of host translocation of PEXEL effectors reported in *Plasmodium*, which involves processing of the PEXEL motif by an endoplasmic reticulum-specific protease followed by the recruitment of the cleaved effectors into a parasite-derived translocon [47–50].

Effector activities: desperately seeking host targets

One critical question in the study of oomycete effectors is the identification of their biochemical activities and mode of action on host cells. The first step towards this objective is to identify the host target molecules of the effectors. So far, the host targets of RXLR effectors have not been described in the literature although it is obvious from conference proceedings that some progress has been made [51]. In contrast, the target proteins of several oomycete apoplastic effectors, many of which function as hydrolase inhibitors, have been determined [35,52–54]. Interestingly, the *P. infestans* cysteine protease inhibitors EPIC1 and EPIC2B bind and inhibit the tomato defense protease Rcr3 [55]. They share this activity with Avr2, an apoplastic effector secreted by the fungus *Cladosporium fulvum* [56]. The finding that unrelated pathogens evolved effectors to inhibit the same host target, suggests that the inhibited protease, Rcr3, is an important component of plant defense [55]. In the future, additional studies on oomycete effectors and their host targets will undoubtedly help to discover and dissect novel plant immune pathways.

3D structures of secreted toxin-like proteins

The structures of two secreted oomycete proteins with toxin activities have been reported recently [57,58]. Several species of bacteria, fungi and oomycetes secrete Nep1-like proteins (NLPs), conserved proteins that have

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evolved or have been acquired by largely unrelated organisms and elicit necrosis and immune responses in the leaves of dicotyledonous plants [59,60]. In *P. infestans* and *P. sojae*, some NLP genes are upregulated during transition from biotrophic to necrotrophic growth [18,61,62]. The crystal structure of an NLP from the oomycete *Pythium aphanidermatum* revealed structural similarity to actinoporins, cytolytic toxins produced by marine organisms [57]. Mutant analyses revealed that the same structural features are required for NLP cytolysis and membrane permeabilization of plant cells, as well as complementation to full virulence of an NLP mutant of the phytopathogenic bacterium *Pectobacterium carotovorum* [57]. These results strengthen the view that NLPs are virulence factors that facilitate *Pythium* and *Phytophthora* nutrient acquisition and thereby colonisation of host plants during the necrotrophic phase of infection [61,62].

PcF is a secreted 52-amino-acid peptide of *Phytophthora cactorum* that contains six 6 cysteines and a 4-hydroxyproline at residue 49 [63,64]. PcF triggers necrosis in tomato and strawberry, an economically important host for *P. cactorum* [64]. PcF is thought to function as a toxin and has multiple homologs in several *Phytophthora* species [18,20,65,66]. Nuclear magnetic resonance (NMR) solution structure of PcF revealed a helix–loop–helix motif fold with similarity to Ole e 6, a major allergen from olive tree pollen suggesting that PcF may mimic plant proteins [58]. However, the exact mode of action of PcF family proteins remains unclear.

Future prospects

The study of oomycetes and their effectors is a highly dynamic and expanding area of research. However, most of the work focuses on a few species, particularly *P. infestans*, *P. sojae* and *H. arabidopsidis*. Little is known about the effectors of other plant parasitic oomycetes, some of which have evolved remarkable adaptations to their parasitic lifestyle (Figure 2). Indeed, the interactions between oomycete parasites and plants can be more complex than colonisation of leaf or root tissue and some species trigger striking symptoms, such as witches brooms (Figure 2g), or specialise on particular plant organs as in the floricolous downy mildews. These observations prompt a number of intriguing questions given that natural selection would favour effectors that mediate any type of phenotypic expression as long as they improve pathogen fitness. Are there oomycete effectors that act beyond altering host immunity? Are there effectors that alter plant development to facilitate pathogen survival and dispersal? Are there effectors that are expressed only in particular plant organs? For instance, downy mildews and white blister rusts that are seed transmitted may have evolved effectors that affect seed survival and germination. Undoubtedly, genome sequencing of a broader spectrum of oomycete species will expand our knowledge of pathogenicity mechanisms and likely reveal novel structural and functional classes of effectors. In particular, it will be interesting to determine the extent to which the knowledge generated on *Phytophthora* effectors will apply to other, more specialised and more biotrophic oomycete pathogens.

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