suggested that the *Ahl* locus is identical to the 'modifier of deaf waddler' (*mdfw*) locus, which is known to interact epistatically with the 'deaf waddler' (*dfw*) mutation on chromosome 6 (Ref. 20). The *dfw* gene (*Atp2b2*) encodes a plasma membrane $Ca^{2+}$-ATPase type 2 pump that has been localized to the stereocilia and basolateral wall of the hair cells in the cochlea[21]. A functional relationship might therefore exist between product of the *Ahl* gene and the ATP-driven $Ca^{2+}$-pump. This mouse model suggests that mild defects of mitochondrial translation and $Ca^{2+}$-pumping, which on their own are harmless, can together produce hearing loss because the lower ATP levels in cells carrying the $tRNA^{Arg}$ mutation are unable to support the impaired $Ca^{2+}$-pump fully.

A pathophysiological mechanism similar to that in the mouse model might lead to maternally inherited non-syndromic deafness in humans. The mouse chromosome 10 region encompassing the *Ahl* locus is syntenic to the human chromosome 10q21–q22 region, making it unlikely that the chromosome 8 modifier of the 1555A→G mutation is the human orthologue of the mouse *Ahl* gene. Although the mouse model does not lead directly to the identification of the human chromosome 8 modifier, the results indicate that genes involved in the ATP-driven ion transport systems of the cochlea are strong potential candidates. It will be interesting to see what the human chromosome 8 modifier turns out to be and at what level it interacts with mutated mitochondrial 12S rRNA.

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**References**

1 Schon, E.A. (2000) Mitochondrial genetics and disease. *Trends Biochem. Sci.* 24, 555–560
2 Kogelnik, A.M. *et al.* (2001) MITOMAP: a human mitochondrial genome database – 1998 update. *Nucleic Acids Res.* 26, 112–115
3 Prezant, T.R. *et al.* (1993) Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat. Genet.* 4, 289–294
4 Bykhovskaya, Y. *et al.* (1998) Evidence for complex nuclear inheritance in a pedigree with nonsyndromic deafness due to a homoplasmic mitochondrial mutation. *Am. J. Med. Genet.* 77, 421–426
5 Estivill, X. *et al.* (1998) Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment with aminoglycosides. *Am. J. Hum. Genet.* 62, 27–35
6 Fischel-Ghodsian, N. (1999) Mitochondrial deafness mutations reviewed. *Hum. Mutat.* 13, 261–270
7 Usami, S. *et al.* (2001) Prevalence of mitochondrial gene mutations among hearing impaired patients. *J. Med. Genet.* 37, 38–40
8 Bykhovskaya, Y. *et al.* (2000) Candidate locus for a nuclear modifier gene for maternally inherited deafness. *Am. J. Hum. Genet.* 66, 1905–1910
9 Hutchin, T. *et al.* (1993) A molecular basis for human hypersensitivity to aminoglycoside antibiotics. *Nucleic Acids Res.* 21, 4174–4179
10 Moazed, D. and Noller, H.F. (1987) Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature* 327, 389–394
11 Gravel, M. *et al.* (1987) Cross-linking of streptomycin to the 16S ribosomal RNA of *Escherichia coli*. *Biochemistry* 26, 6227–6232
12 Chambers, H.F. and Sande, M.A. (1996) The aminoglycosides. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (9th edn), (Hardman, J.G. *et al.*, eds), pp. 1103–1121, McGraw–Hill
13 Hamasaki, K. and Rando, R.R. (1997) Specific binding of aminoglycosides to a human rRNA construct based on a DNA polymorphism which causes aminoglycoside-induced deafness. *Biochemistry* 36, 12323–12328
14 Guan, M-X. *et al.* (1996) Biochemical evidence for nuclear gene involvement in phenotype of non-syndromic deafness associated with mitochondrial 12S rRNA mutation. *Hum. Mol. Genet.* 5, 963–971
15 Guan, M-X. *et al.* (2000) A biochemical basis for the inherited susceptibility to aminoglycoside ototoxicity. *Hum. Mol. Genet.* 9, 1787–1793
16 Guan, M-X. *et al.* (2001) Nuclear background determines biochemical phenotype in the deafness-associated mitochondrial 12S rRNA mutation. *Hum. Mol. Genet.* 10, 573–580
17 Taanman, J-W. (1999) The mitochondrial genome: structure, transcription, translation and replication. *Biochim. Biophys. Acta* 1410, 103–123
18 O'Brien, T.W. *et al.* (2000) Mammalian mitochondrial ribosomal proteins (4). Amino acid sequencing, characterization, and identification of corresponding gene sequences. *J. Biol. Chem.* 275, 18153–18159
19 Johnson, K.R. *et al.* (2001) A nuclear–mitochondrial DNA interaction affecting hearing impairment in mice. *Nat. Genet.* 27, 191–194
20 Zheng, Q.Y. and Johnson, K.R. (2001) Hearing loss associated with the modifier of deaf waddler (*mdfw*) locus corresponds with age-related hearing loss in 12 inbred strains of mice. *Hearing Res.* 154, 45–53
21 Street, V.A. *et al.* (1998) Mutations in a plasma membrane $Ca^{2+}$-ATPase gene cause deafness in deafwaddler mice. *Nat. Genet.* 19, 390–394

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# Genetics and genomics of the oomycete–host interface

Brett M. Tyler

**Oomycetes are phylogenetic relatives of brown algae that cause many destructive diseases of plants, as well as several animal and human diseases. Because of oomycetes' distinct physiology, most fungicides are ineffective against them. With the aid of genetic and genomic tools, oomycete genes encoding secreted proteins that control the outcome of infection are being identified. Ongoing genomics efforts promise to identify further genes and create the possibility of new control measures.**

Oomycetes resemble fungi, both morphologically and physiologically (Box 1), but they are actually phylogenetic cousins of diatoms and brown algae, within the kingdom Stramenopiles (Fig. 1, Box 1). They are as distant from fungi, plants and animals as are ciliates (e.g. *Paramecium*) and apicomplexan parasites (*Plasmodium*, which causes malaria)[1]. Herein lies a severe problem: the oomycetes include many destructive pathogens of plants, animals and humans, and many of the tools mankind has developed to protect itself against fungi fail when confronted by oomycetes.

## An algal scourge

Oomycetes inflict their greatest damage on plants. Most of the 60 or so species of the oomycete genus *Phytophthora* and most of the 100 or more species of the closely related genus *Pythium* are destructive plant pathogens[2]. *Phytophthora infestans*, which causes the late-blight disease of potato, destroyed the Irish potato crop in 1845 and 1846, resulting in the Irish potato

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**Box 1. Similarities between fungi and oomycetes – convergent evolution?**

Sequences of conserved genes such as 18S rRNA (Fig. 1), actin and tubulin show clearly that fungi and oomycetes are phylogenetically distinct. This is also reflected in key physiological distinctions. Oomycete cell walls contain cellulose, whereas those of fungi contain chitin or chitosan. Oomycetes synthesize lysine via diamino-pimelate, whereas fungi synthesize it via α-amino-adipate. Nevertheless, there are many intriguing similarities, the most prominent being the hyphal growth habit and the variety of spores adapted to aerial dispersal (conidia), water dispersal (zoospores) and long-term persistence (chlamydospores). Both oomycetes and fungi are heterotrophic, with a vast array of enzymes for acquiring carbon, nitrogen, sulfur and other essential nutrients from diverse sources. These common features suggest convergent evolution as the two groups have adapted to similar ecological niches. Oomycete and fungal plant pathogens also share similarities; for example, the differentiation of appressoria for breaching physical barriers erected by plants. Both groups of pathogens also span a full range of pathogenic strategies from aggressive necrotrophs through to highly evolved obligately parasitic biotrophs. Assuming such similarities also reflect convergent evolution, driven by the need to overcome plant defenses, identifying similarities in the pathogenic mechanisms of oomycetes and fungi at the molecular level could enable key functions essential for a successful plant pathogen to be identified.

famine. This is still a damaging disease, annually costing over $5 billion worldwide in crop losses and control measures. Several species of *Phytophthora* cause severe losses to cacao growers, especially in West Africa and Brazil, and threaten the world's chocolate supply. *Phytophthora cinnamomi*, which can infect nearly 2000 different plant species, has caused severe damage to forests in Australia, Europe and the USA. Oomycete animal pathogens are primarily aquatic, affecting insects, crustaceans, fish and amphibians[3], but some, such as *Pythium insidiosum*, cause dangerous infections of humans[4].

Because of oomycetes' distinct physiology, many of the most effective fungicides fail against them. For example, the azole fungicides, which are used extensively in agriculture and medicine, target ergosterol biosynthesis. However, oomycetes do not synthesize sterols but acquire them from their victims[2]. A further complication is that many oomycetes appear to have an extraordinary genetic flexibility that enables them to adapt rapidly to and overcome chemical control measures and genetic resistance bred into plant hosts[2]. Resistance to effective chemicals such as metalaxyl has arisen in several oomycete species against which they have been deployed, and careful management is required to preserve the usefulness of the chemicals[5]. Plants bred with genetic resistance against *P. infestans* remained resistant for less than a year[6].

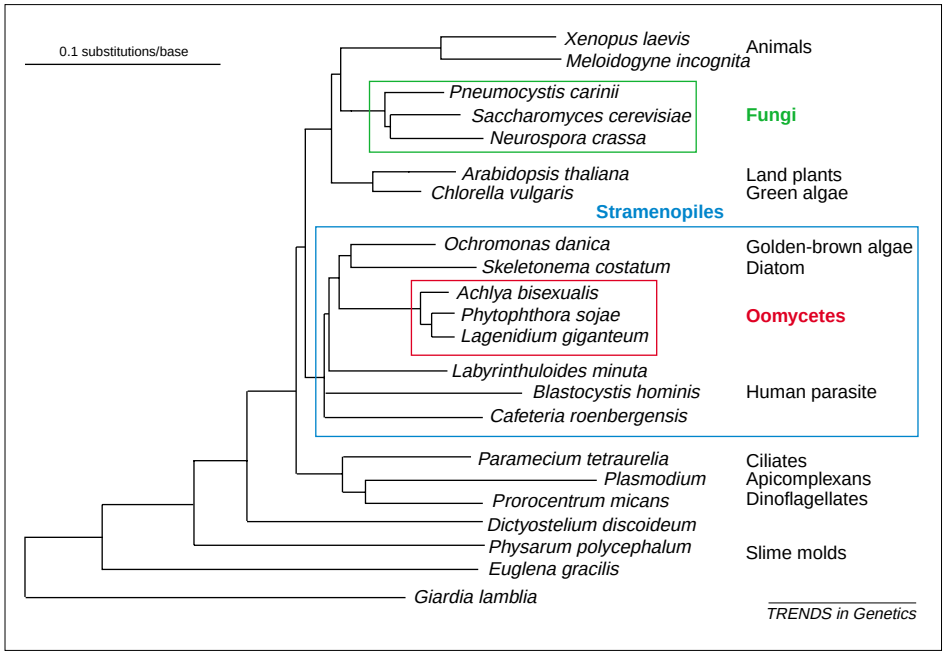


Fig. 1. Oomycetes are not fungi: eukaryotic phylogeny based on 18S ribosomal RNA sequences. Adapted from Ref. 1.

The destructiveness of oomycete diseases, and the difficulty of controlling them, has led to a concerted effort to develop molecular genetic and genomic tools to investigate these organisms. Due to the economic impact and genetic amenability of *P. infestans* and *Phytophthora sojae* (a soybean pathogen), these efforts are most advanced in these two *Phytophthora* species. More recently, model plant pathogens such as *Phytophthora porri* (on *Arabidopsis*), *Phytophthora medicaginis* (on *Medicago truncatula*) and *Phytophthora palmivora* (on *Nicotiana benthamiana*) have begun to attract interest. Detailed genetic maps have been constructed for *P. sojae*[7] and *P. infestans*[8], primarily using molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) (Fig. 2). A genetic map has also been constructed for the obligate pathogen *Bremia lactucae* (lettuce downy mildew)[9]. Transformation systems, including gene silencing[10,11], have been developed for several *Phytophthora* species, using a variety of technologies including protoplast fusion and particle bombardment (reviewed in Ref. 12), and, more recently, electroporation of zoospores lacking cell walls (B. Tyler and F. Govers, unpublished) and *Agrobacterium* transformation (I. Vijn and F. Govers, unpublished).

**Proteins at the *Phytophthora*–plant interface**

Most of the genetic studies have focused on genes that plant pathologists call 'avirulence' genes. Avirulence genes encode products that are detected by plants' defense systems, specifically by receptors encoded by so-called major resistance genes[13] (Fig. 3). Avirulence genes are of interest because in bacterial plant pathogens, many of these genes encode proteins that are injected directly into host cells by a specialized secretion system, presumably to disable the defense machinery of the host[14]. It seems plausible, therefore, that some avirulence genes from oomycetes will also encode proteins designed to paralyze their hosts. Cloning

avirulence genes, therefore, is a good way of understanding not only how plants defend themselves against oomycetes, but also how oomycete pathogens attack plants. Bacterial pathogens of animals and humans also inject avirulence-like proteins into their host cells[14], and oomycete pathogens of these hosts might do likewise.

Ten avirulence genes have been placed on the genetic map of *P. sojae*[7,15] and six on the map of *P. infestans*[16] (Fig. 2). In contrast to avirulence genes in true fungi, many avirulence genes in oomycetes are clustered. In *P. sojae*, two sets of co-segregating genes (*Avr1b*/*Avr1k*, *Avr4*/*Avr6*), and two closely linked genes (*Avr3a* and *Avr5*) have been observed[7,17]. In *P. infestans*, *Avr3*, *Avr10* and *Avr11* are clustered[16]. At the recent Fungal Genetics Conference in Asilomar, California, several laboratories reported progress in map-based cloning of these avirulence genes. Bacterial artificial chromosome (BAC) contigs spanning the *Avr1a* (T. MacGregor and M. Gijzen, Agriculture Canada, London, UK) and the *Avr11* and *Avr4* loci of *P. infestans* (S.Whisson and D. Birch, Scottish Crops Research Institute, Invergowrie, UK; T. van der Lee and F. Govers, Wageningen University, the Netherlands; Ref. 18) have been identified. Cosmid contigs spanning the *Avr4*/*Avr6* locus of *P. sojae* have also been obtained (S. Whisson *et al.*, pers. commun.).

Our laboratory has identified a BAC contig spanning the *Avr1b*/*Avr1k* locus of *P. sojae*, and we have identified two genes on the contig required for the Avr1b phenotype (W. Shan and B. Tyler, unpublished). *Avr1b*-1 encodes a small secreted protein that triggers a defense response in soybean plants containing the appropriate major resistance gene (*Rps1b*), whereas *Avr1b*-2 is required for the transcription of *Avr1b*-1. The *Avr1b*-1 gene is transcribed in the pathogen only during infection, and the protein appears to spread systemically through the plant, supporting the notion that it could contribute to the ability of the pathogen to attack its host. Elicitins, conserved proteins secreted by all *Phytophthora* species, act as avirulence factors in the interaction between *Phytophthora* and *Nicotiana* species[19,20]; the genes for different elicitin isoforms are also clustered (Ref. 21; R. Jiang and F. Govers, unpublished). The clustering of avirulence genes in oomycetes suggests that, as in many pathogens of plants and animals, oomycete genes involved in infection might be clustered in pathogenicity islands.

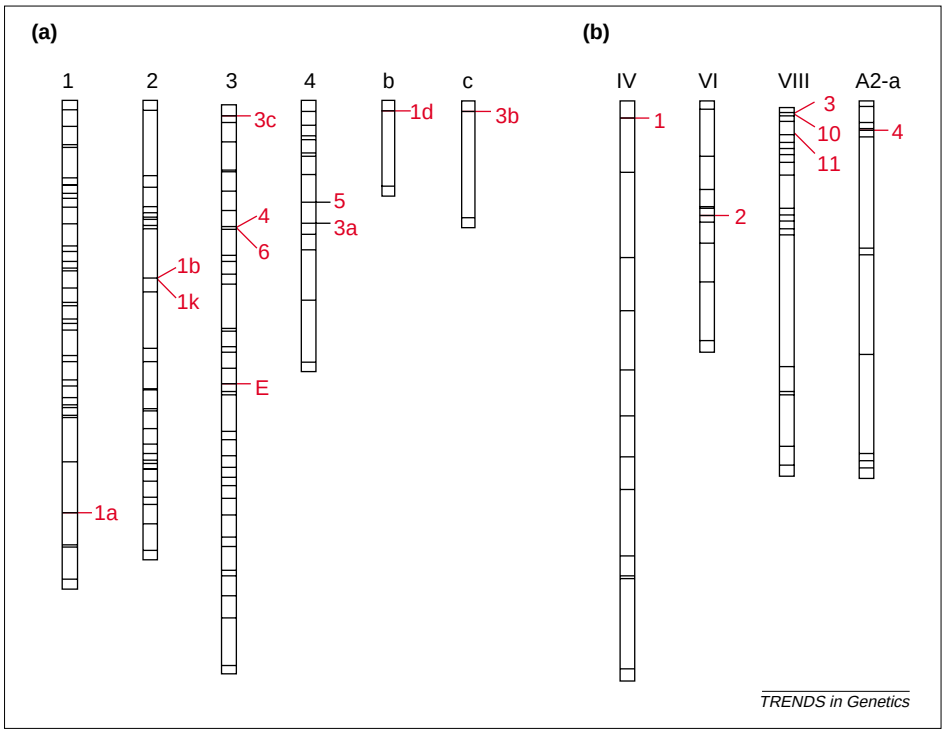


Fig. 2. Genetic maps of *Phytophthora sojae* and *Phytophthora infestans*. (a) Four of 21 major linkage groups of *P. sojae* and two of seven minor groups containing avirulence genes[7,15] are shown. (b) Three of ten major linkage groups of *P. infestans* and one of seven minor groups containing avirulence genes[16] are shown. In both (a) and (b), 1 indicates *Avr1*; 1a, *Avr1a*; etc.; E indicates the elicitin genes. The positions of molecular markers on the maps are denoted by horizontal lines.

**Genomics identifies many new candidates**

Several genomic projects are actively underway in *P. sojae* and *P. infestans*. *Phytophthora* researchers have formed the *Phytophthora* Genome Initiative to coordinate and promote *Phytophthora* genomics (http://www.ncgr.org/pgc). Several thousand expressed sequence tags (ESTs) of each species are available in public databases (http://www.ncgr.org/pgc), and a project to develop 41 000 additional *P. sojae* ESTs and 14 000 *P. infestans* ESTs was funded last year by the USDA. In addition, 35 000 *P. infestans* ESTs developed by an international consortium funded by Syngenta are expected to become publicly available in 2003.

At the 2001 Fungal Genetics Conference, S. Kamoun (Ohio State University, Columbus, OH, USA) described a high-throughput strategy for identifying secreted *P. infestans* proteins from the EST database that influence infection. Proteins encoded by the ESTs were screened for secretory peptide leaders using the signalP algorithm. One of the ESTs identified in this manner proved to be a partially conserved homolog of the *P. sojae Avr1b-1* gene, showing that the procedure could indeed pick out proteins important for infection. ESTs of interest were expressed in the host plants *Nicotiana benthamiana* and tomato using a systemic viral expression system, then assayed for changes in susceptibility to *Phytophthora* infection. Several cDNAs were identified that induce necrosis in plant tissue and alter the tomato response to *P. infestans*. D. Qutob (Agriculture Canada), M. Gijzen and S. Kamoun used this approach to screen *P. sojae* ESTs, identifying a protein that triggers a defense response in a wide variety of plants. ESTs encoding the protein, originally described as a necrosis- and ethylene-inducing peptide, also occur in *P infestans*, *Phytophthora parasitica*, *P. medicaginis* and *Pythium aphanidermatum*. Intriguingly, the protein is also found in true fungi and in bacteria, but only in a very small subset of sequenced genomes, suggesting that it spread among these kingdoms by horizontal gene transfer.

The next major goal in *Phytophthora* genomics is a complete genome sequence. The genome sizes of *P. sojae* and *P. infestans* are 62 Mb and 250 Mb respectively, so the focus has been on

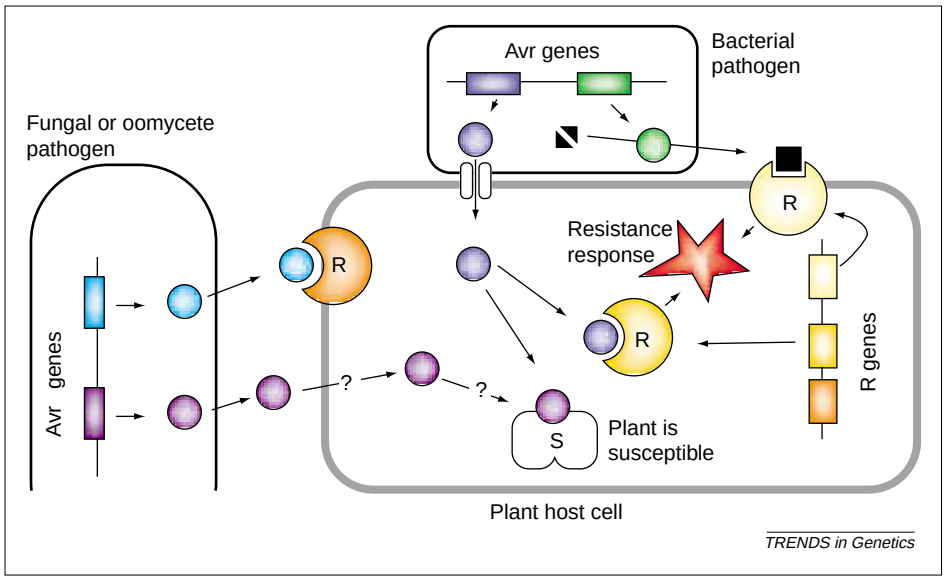


Fig. 3. The role of avirulence genes in plant–pathogen interactions. Some avirulence (*Avr*) genes encode proteins (purple and blue) that are secreted from bacterial, fungal or oomycete pathogens. Other *Avr* genes (green) encode enzymes responsible for synthesis or export of small molecular weight molecules (black) secreted from the cell. Many bacterial avirulence proteins (dark purple) enter host cells through a specialized apparatus, the type III secretion system[14]. There is only indirect evidence that fungal and oomycete Avr proteins (light purple) enter the plant cell (e.g. Ref. 23), and no mechanism has been identified. The direct or indirect products of *Avr* genes interact with intracellular or membrane receptors encoded by resistance genes (R; yellow and orange), triggering an effective defense response. The receptor–ligand relationship of R gene and *Avr* gene products means that individual R genes confer resistance only when the cognate *Avr* gene is present in the pathogen, termed the 'gene-for-gene' relationship. Plants typically have hundreds of R genes to protect themselves against diverse pathogens. It is hypothesized that when an R gene with the correct specificity is missing, *Avr* gene products can interact with other components of the cell (S), disabling its defense machinery and rendering the plant susceptible to infection.

*P. sojae*. The genomes of other oomycetes are of comparable size to *P. sojae*[22]. Comparative genomics studies are just beginning. *P. sojae* and *P. infestans* displayed microsynteny in one 100-kb region near *Avr1b*-1 (R. Jiang, B. Tyler and F. Govers, unpublished). In preparation for genome sequencing, our group has partially assembled the *P. sojae* BAC library into contigs by hybridization fingerprinting. The contig will be completed by restriction enzyme fingerprinting and by high-throughput hybridization with ESTs.

Results to date show that repetitive sequences (about 50% of the *P. sojae* genome) are highly clustered, and at least a third of the *P. sojae* genome appears to be largely free of repetitive sequences and rich in genes, based on hybridization with EST clones. Sequencing of one 62-kb BAC near *Avr1b-1* has revealed that *P. sojae* genes are packed into dense clusters separated by longer regions of noncoding sequences. Within the clusters, average spacing between genes is less than 300 base pairs, and three examples of overlapping genes occur within just this one BAC. Complete sequencing of the *P. sojae* genome is estimated to cost $6–8 million, and vigorous efforts to raise these funds in the public and private sectors are underway in several countries, especially the USA. A complete sequence of *P. sojae* will greatly aid research into other oomycete species through comparative genomics.

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**References**

1 Sogin, M.L. and Silberman, J.D. (1998) Evolution of the protists and protistan parasites from the perspective of molecular systematics. *Int. J. Parasitol.* 28, 11–20
2 Erwin, D.C. and Ribiero, O.K. (1996) *Phytophthora Diseases Worldwide*, APS Press
3 Wong, M.K.M. *et al*. (1998) Role of fungi in freshwater ecosystems. *Biodiversity Conserv.* 7, 1187–1206
4 Kaufman, L. (1998) Penicilliosis marneffei and pythiosis: emerging tropical diseases. *Mycopathologia* 143, 3–7
5 Gisi, U. and Cohen, Y. (1996) Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. *Annu. Rev. Phytopathol.* 34, 549–572
6 Fry, W.E. (1982) Principles of plant disease management, Academic Press
7 Whisson, S.C. *et al*. (1995) *Phytophthora sojae* avirulence genes, RAPD and RFLP markers used to construct a detailed genetic linkage map. *Mol. Plant–Microbe Ints.* 8, 988–995
8 van der Lee, T. *et al*. (1997) AFLP linkage map of the oomycete *Phytophthora infestans*. *Fungal Genet. Biol.* 21, 278–291
9 Hulbert, S.H. *et al*. (1988) Genetic analysis of the fungus *Bremia lactucae*, using restriction fragment length fragment polymorphisms. *Genetics* 120, 947–958
10 Judelson, H.S. *et al*. (1993) Expression and antisense inhibition of transgenes in *Phytophthora infestans* is modulated by choice of promoter and position effects. *Gene* 133, 63–69
11 Van West, P. *et al*. (1999) Internuclear gene silencing in *Phytophthora infestans*. *Mol. Cell* 3, 339–348
12 Judelson, H.S. (1997) The genetics and biology of *Phytophthora infestans*: Modern approaches to a historical challenge. *Fungal Genet. Biol.* 22, 65–76
13 Lauge, R. and De Wit, P.J.G.M. (1998) Fungal avirulence genes: Structure and possible functions. *Fungal Genet. Biol.* 24, 285–297
14 Gabriel, D.W. (1999) Why do pathogens carry avirulence genes? *Physiol. Molec. Plant Path.* 55, 205–214
15 May, K.J. *et al*. Inheritance and mapping of eleven avirulence genes in *Phytophthora sojae*. *Fungal Genet. Biol.* (in press)
16 van der Lee, T. *et al*. (2001) Mapping of avirulence genes in *Phytophthora infestans* with amplified fragment length polymorphism markers selected by bulked segregant analysis. *Genetics* 157, 949–956
17 Gijzen, M. *et al*. (1995) Cosegregation of *Avr4* and *Avr6* in *Phytophthora sojae*. *Can. J. Bot.* 74, 800–802
18 Whisson, S.C. *et al*. Physical mapping across an avirulence locus of *Phytophthora infestans* using a high representation, large insert bacterial artificial chromosome library. *Mol. Genet. Genomics* (in press)
19 Kamoun, S. *et al*. (1994) Potential role of elicitins in the interaction between *Phytophthora* species and tobacco. *Appl. Environ. Microbiol.* 60, 1593–1598
20 Kamoun, S. *et al*. (1998) Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. *Plant Cell* 10, 1413–1426
21 Panabieres, F. *et al*. (1995) Characterization of a gene cluster of *Phytophthora cryptogea* which codes for elicitins, proteins inducing a hypersensitive-like response to tobacco. *Mol. Plant–Microbe Interact.* 8, 996–1003
22 Voglmayr, H. and Greilhuber, J. (1998) Genome size determination in Peronosporales (Oomycota) by Feulgen image analysis. *Fungal Genet. Biol.* 25, 181–195
23 Jia, Y. *et al*. (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J.* 19, 4004–4014

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