MPMI Vol. 9, No. 4, 1996, pp. 305–309. Publication no. M-1996-0229-01N.

Research Note

Presence of *Ty1-Copia* Group Retrotransposon Sequences in the Potato Late Blight Pathogen *Phytophthora infestans*

Paul W. Tooley[1] and David J. Garfinkel[2]

[1]USDA-ARS, Foreign Disease-Weed Science Research, Ft. Detrick, Frederick, MD 21701, U.S.A.; and [2]Gene Regulation and Chromosome Biology Laboratory, NCI-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, MD 21702-1201, U.S.A.

Received 31 May 1995. Accepted 7 February 1996.

Multiple copies of retrotransposon reverse transcriptase coding sequences were identified in *Phytophthora infestans* by polymerase chain reaction (PCR) amplification using degenerate primers. The *P. infestans* sequences belong to the Ty1-copia superfamily, and putative elements from different *P. infestans* isolates show restriction site polymorphisms. Some contain complete open reading frames while others do not, indicating the presence of potentially active as well as inactive elements.

Additional keywords: potato late blight, transposable element.

Few genetic mechanisms have been elucidated to explain the high degree of pathogenic variation long noted within *Phytophthora infestans* (Mont.) de Bary, causal agent of potato late blight. High rates of evolution of new, virulent, fungicide-resistant phenotypes suggest that transposable element activity may play a role. Transposons and retroelements have recently been described in diverse genera of filamentous fungi; to date, most have belonged to the *gypsy* group of LTR retrotransposons (Oliver 1992; Garfinkel 1992). LTR retro-elements within the *Ty1-copia* group, although found in a diverse array of organisms (Flavell 1992) have not, to our knowledge, been identified in filamentous fungi. The genus *Phytophthora* (a member of the class Oomycetes) is placed taxonomically in the same group as slime molds, certain algae, and protozoans, and is not considered a "true" fungus (Margulis et al. 1989). Thus, if *Phytophthora* species contain transposable elements they might be expected to be different from those described in other filamentous fungi. Indeed, the *Tp1* element of the slime mold *Physarum polycephalum*, whose sequence family constitutes over half of the organism's repetitive DNA fraction, has been found to belong to the *Ty1-copia* group (Rothnie et al. 1991).

Reverse transcriptase is required for replication of retroviruses and retroviral-like elements and is present in numerous organisms (Xiong and Eickbush 1990). To determine whether reverse transcriptase sequences and hence retroviral-like transposable elements are present in *P. infestans*, we employed primers successful in amplifying reverse transcriptase sequences of the *Ty1-copia* superfamily from plants

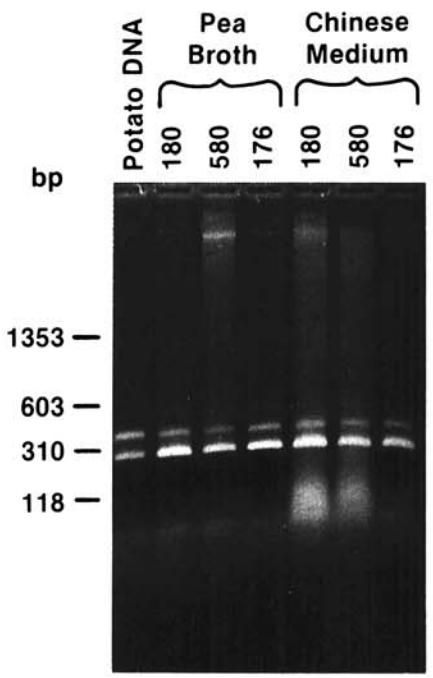

Fig. 1. Polymerase chain reaction (PCR) products obtained from DNA of three isolates of *Phytophthora infestans*. PCR reactions were carried out in a Perkin-Elmer Cetus (Norwalk, CT) model 9600 thermal cycler programmed for 30 cycles of 94°C for 15 s, 45°C for 15 s, and 72°C for 15 s, followed by a 6 min extension at 72°C. Reactions contained 1 U *Taq* polymerase (Perkin-Elmer Cetus), 50 pM each primer, 100 μM deoxynucleoside triphosphates, and 1.5 mM $MgCl_2$ in 25-μl reaction volume with no oil overlay. **A,** Primers (ACNGCNTTYYTNCAYGG and ARCATRTCRTCNACRTA, where Y = C + T, R = A + G, and N = A + C + G + T) were as described in Flavell et al. (1992b). PCR products obtained from *P. infestans* were identical in size to products obtained when potato DNA was used as template (first lane). DNA was extracted from mycelium grown on pea broth as well as on synthetic (defined) medium (Xu et al. 1982).

Corresponding author: Paul W. Tooley;
E-mail: ptooley@asrr.arsusda.gov

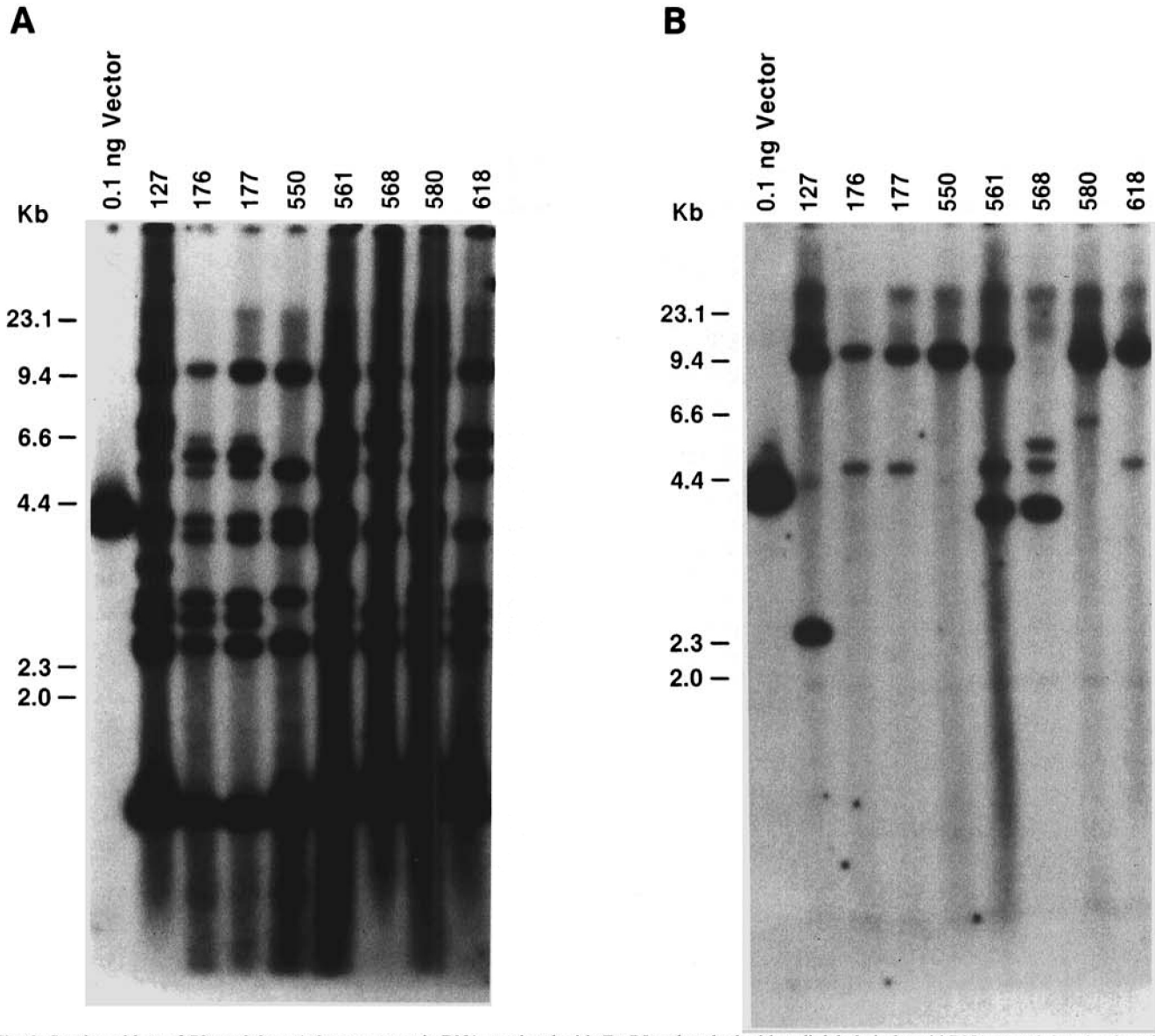

Fig. 2. Southern blots of *Phytophthora infestans* genomic DNA restricted with *Eco*RI and probed with radiolabeled plasmid DNA containing **A**, cloned retroviral-like element PI580-4 and **B**, cloned element PI580-1. Element PI580-4 appears to be present in higher copy number in the *P. infestans* genome compared with element PI580-1. With both elements, variation was observed among *P. infestans* isolates, illustrating potentially useful polymorphisms. Isolate sources are described elsewhere (Deahl et al. 1991; Tooley et al. 1985).

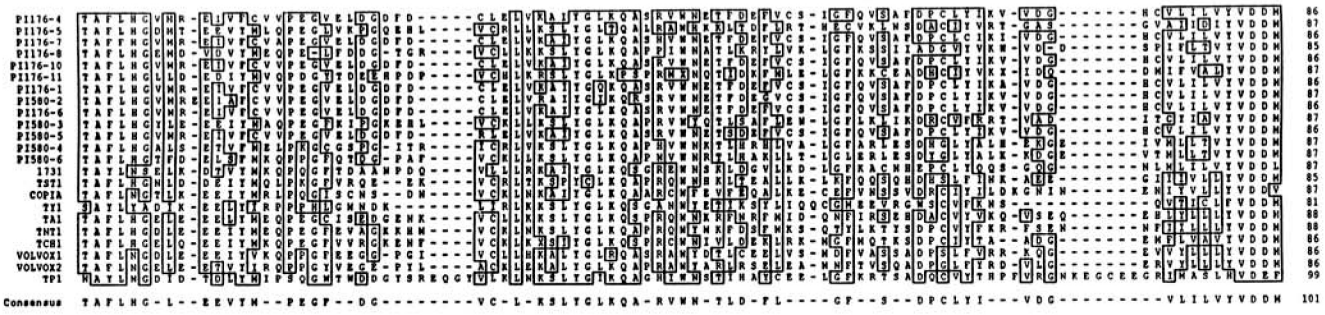

Fig. 3. Amino acid sequence alignment of *Ty1-copia* element reverse transcriptase sequences from *Phytophthora infestans* and other organisms. Sequences were analyzed using the UW-GCG package of programs (Devereaux et al. 1984) and aligned using CLUSTAL W (Thompson et al. 1994). Boxes delineate residues present in the consensus sequence, which is shown at the bottom of the figure. *Phytophthora infestans* sequences are represented by designations PI176-4, PI580-2, etc. Other *Ty1-copia* group sequences included in the alignment are as follows: *1731* and *copia* (*Drosophila melanogaster*), *Tst1* (*Solanum tuberosum*), *Ty1* (*Saccharomyces cerevisiae*), *Ta1* (*Arabidopsis thaliana*), *Tnt1* (*Nicotiana tabacum*), *Tch1* (*Clupea harengus*), *Volvox1* and *Volvox2* (*Volvox carteri*), and *Tp1* (*Physarum polycephalum*) (Camirand and Brisson 1990; Flavell and Smith 1992; Rothnie et al. 1991; Voytas et al. 1992; Xiong and Eickbush 1990).

(Flavell et al. 1992a, 1992b) and vertebrates (Flavell and Smith 1992; Flavell et al. 1995).

Isolates were chosen to represent the range of genetic diversity present within *P. infestans* (Deahl et al. 1991; Goodwin et al. 1992; Tooley et al. 1985). Isolates were grown on defined medium (Xu et al. 1982) to avoid potential polymerase chain reaction (PCR) amplification from plant products present in some standard fungal culture media such as V8 juice, pea broth, or rye seed medium. DNA was extracted using the methods described by Goodwin et al. (1992), and DNA hybridizations were performed using standard methods (Amasino 1986; Sambrook et al. 1989). Primers used for PCR span reverse transcriptase domain 4 (Xiong and Eickbush 1990) and contain the reverse transcriptase active site coding region (Kohlstaedt et al. 1993).

Two PCR products (260 and 302 bp) were obtained from DNA of *P. infestans* isolates 176, 180, and 580; identical products were obtained when template DNA was extracted from fungal mycelium grown on pea broth, or on Chinese synthetic (defined) medium (Fig. 1). PCR products were cloned into appropriate vectors and given the designations PI176-1, PI176-2, etc., and PI580-1, PI580-2, etc., to indicate clones from isolates 176 and 580, respectively. Clones PI580-1 (302 bp product) and PI580-4 (260 bp product) were used to probe Southern blots of total genomic DNA of eight isolates of *P. infestans* restricted with *Eco*RI (Fig. 2). Clone PI580-4 (Fig. 2A) hybridized with 7 to 10 bands for the eight *P. infestans* isolates chosen, while clone PI580-1 hybridized with 1 to 3 bands (Fig. 2B). Variation in band intensity within isolates and clear polymorphisms among isolates were observed (Fig. 2). There were no obvious correlations noted between banding patterns and mating type or geographic origin of the isolates.

DNA sequences obtained from eight clones from isolate 176 and six clones from isolate 580 were translated into protein sequences using the UW-GCG TRANSLATE program. The correct frame and orientation of each coding sequence was determined by the presence of the primer sequences on each end of the coding sequence. Open reading frames (ORFs) present in each sequence were identified and protein

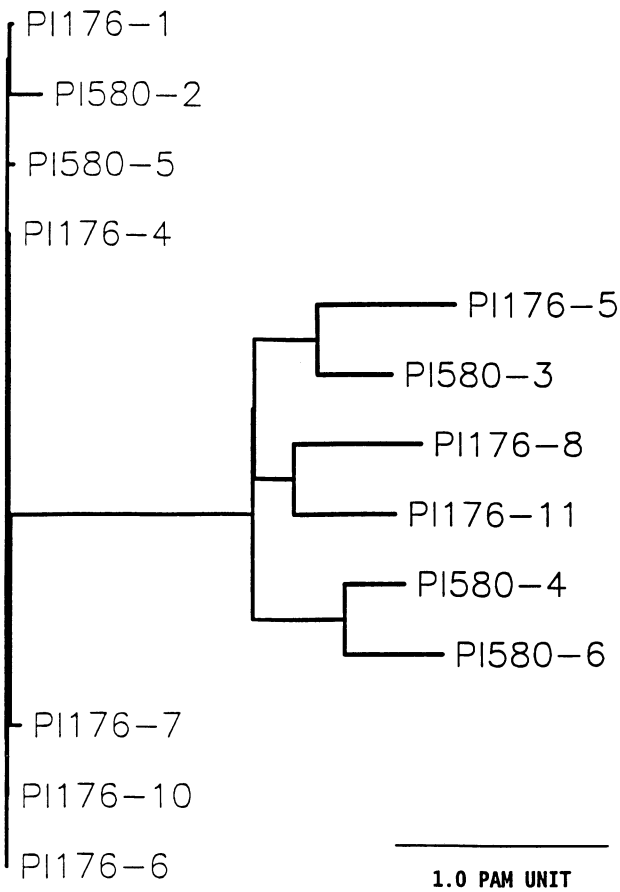

Fig. 4. Dendrogram produced in PHYLIP using neighbor-joining (Felsenstein 1993; Saitou and Nei 1987), showing relationships among the 13 *Phytophthora infestans* reverse transcriptase sequences (eight from isolate 176 and five from isolate 580). Seven sequences were identical or very similar to one another. Distances are measured on the horizontal axis only, in PAM units (Dayhoff et al. 1978; Felsenstein 1993). Primer sequences were removed for analyses of sequence relatedness.

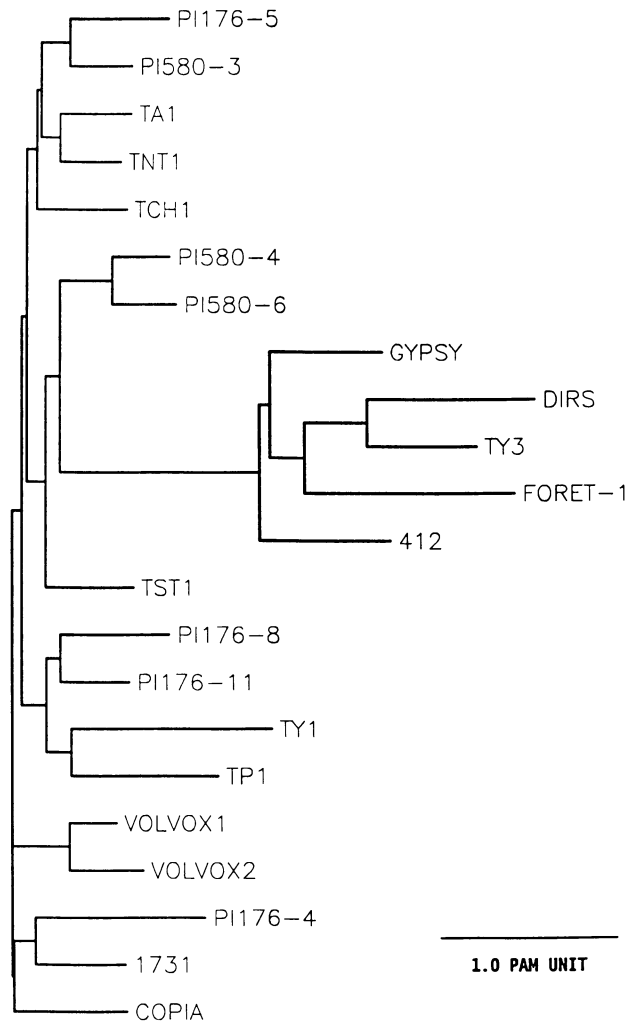

Fig. 5. Dendrogram showing clustering relationships among *Phytophthora infestans*, *Ty1-copia,* and *gypsy* group reverse transcriptase sequences. See Figure 3 caption for abbreviations of *Ty1-copia* group members. The *gypsy* elements shown are as follows: *gypsy* and *412* (*Drosophila melanogaster*), *Ty3* (yeast), *Foret-1* (*Fusarium oxysporum*), and *Dirs* (*Dictyostelium discoideum*) (Flavell and Smith 1992; Julien et al. 1992; Xiong and Eickbush 1990). Distances are in PAM units (Dayhoff et al. 1978; Felsenstein 1993).

sequences were scanned for presence of the conserved *Ty1-copia* group motif SLYXLKQAXRXW (Flavell et al. 1992b; Flavell et al. 1995; Xiong and Eickbush 1990). The single clone (PI580-1) that represented the higher molecular weight (302 bp) PCR product was found to contain portions of the conserved motif. However, it had the same primer sequence on each end, indicating that it may be a cloning artifact or the result of amplification with only one of the primers; it thus was not included in further analysis. Of the remaining 13 clones (eight from isolate 176 and five from isolate 580), eight contained full-length ORFs and five contained short ORFs and stop codons.

Multiple sequence alignments were performed using the five non-full-length ORF sequences and the eight sequences with complete ORFs to determine the location of frameshifts. Non-full-length ORF sequences were "corrected" by simple sequence editing where possible to facilitate multiple alignment and construction of dendrograms indicating sequence relationships. However, some of the sequences may represent inactive transposons and thus would not be expected to contain ORFs or be otherwise functional. For clones PI176-6, PI580-2, PI580-4, and PI580-6, an extra base was added or subtracted from the sequence to put it in frame and eliminate stop codons. For sequence PI176-11, it was not possible to produce a full-length ORF by simple sequence editing, and it thus contains two stop codons (represented by Xs in the sequence).

Amino acid sequences from *P. infestans* were aligned with those of other *Ty1-copia* group members including the photosynthetic protist *Volvox carteri* and the slime mold *Physarum polycephalum* (Fig. 3). The *P. infestans* sequences are clearly members of the *Ty1-copia* retrotransposon group due to the presence of the consensus motif SLYXLKQAXRXW in some sequences, and very similar motifs in others (Fig. 3).

Sequences PI176-1, PI176-4, PI176-6, PI176-7, PI176-10, PI580-2, and PI580-5 were nearly identical (Fig. 4) with percent similarities (measures of exact amino acid matches plus conserved substitutions) ranging from 93 to 100%. These sequences clustered separately from sequences PI176-5, PI176-8, PI176-11, PI580-3, PI580-4, and PI580-6, which showed substantial variation (Fig. 4). Percent similarities among all of the *P. infestans* sequences ranged from 47 to 100%.

A dendrogram was constructed to show relationships among various *Ty1-copia* group elements including the *P. infestans* elements (Fig. 5). To avoid redundancy, only one sequence (PI176-4) was chosen from the large cluster of seven very similar *P. infestans* sequences (Fig. 4), along with the additional six less closely related *P. infestans* sequences. Also included were 10 members of the *Ty1-copia* group as well as five members of the *gypsy* group of retroelements. The *gypsy* elements clustered separately from the *Ty1-copia* elements (Fig. 5). Some of the *P. infestans* sequences showed closer relationships with other *Ty1-copia* group elements than with other *P. infestans* elements. For two *P. infestans* sequences (PI176-4 and PI580-6), closest relationships (i.e., minimum or near-minimum distances) were observed with a sequence from the alga *Volvox carteri* (VOLVOX1), while for other *P. infestans* sequences (PI176-5, PI176-8, PI176-11, PI580-3, and PI580-4) such relationships were observed with elements *Tnt1* from tobacco and/or element *Ta1* from *Arabidopsis*. No consistent pattern of similarity was observed between *P. infestans* sequences and those of specific other *Ty1-copia* group members, such as the alga *Volvox carteri* or the slime mold *Physarum polycephalum*.

Flavell (1992) has suggested that horizontal transmission of *Ty1-copia* sequences between different species has occurred in the past and contributed to their evolution. Our data may support the horizontal transmission hypothesis in that sequences from *P. infestans* and its plant host, *Solanum tuberosum* L. (element *Tst1*), showed percent similarities ranging from 51 to 64%, which exceeds some of the percent similarities observed among *P. infestans* elements (range 47 to 100%).

Variation in the intensity of specific bands observed within isolates such as 176 and 177 on Southern blots may indicate that a portion of a given element may be present in low copy number while other portions may be highly repeated. It is also possible that multiple *Ty1-copia* families may exist within *P. infestans* and that multiple bands may reflect different types of elements within a given isolate. Additional characterization of full-length elements will be required to determine whether retroelements contribute to variation in pathogenicity and other characters in *P. infestans*.

ACKNOWLEDGMENTS

We would like to thank Marie Carras for excellent technical assistance and Bill Modi and Mike Smith for assistance and valuable discussions on protein sequence analysis. Jeff Strathern provided valuable input at several stages of this research. We also thank Gary Smythers and Mark Gunnell of the Frederick Biomedical Supercomputing Center for their help with computer analyses. This research was sponsored, in part, by the National Cancer Institute under contract NO1-CO-46000 with ABL.

LITERATURE CITED

Amasino, R. M. 1986. Acceleration of nucleic acid hybridization rate by polyethylene glycol. Anal. Biochem. 152:304-307.

Camirand, A., and Brisson, N. 1990. The complete nucleotide sequence of the Tst1 retrotransposon of potato. Nucleic Acids Res. 18:4929.

Dayhoff, M. O., Schwartz, R. M., and Orcutt, B. C. 1978. A model of evolutionary change in proteins. Pages 345-352 in: Atlas of Protein Sequence and Structure. M. O. Dayhoff, ed. National Biomedical Research Foundation, Washington, DC.

Deahl, K. L., Goth, R. W., Young, R., Sinden, S. L., and Gallegly, M. J. 1991. Occurrence of the A2 mating type of *Phytophthora infestans* in potato fields in the United States and Canada. Am. Potato J. 68:717-725.

Devereaux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12: 387-395.

Felsenstein, J. 1993. PHYLIP Phylogeny Inference Package User's Guide. Frederick Biomedical Supercomputer Center, Frederick, MD.

Flavell, A. J. 1992. *Ty1-copia* group retrotransposons and the evolution of retroelements in the eukaryotes. Genetica (The Hague) 86:203-214.

Flavell, A. J., Dunbar, E., Anderson, R., Pearce, S. R., Hartley, R., and Kumar, A. 1992a. *Ty1-copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucleic Acids Res. 20:3639-3644.

Flavell, A. J., Jackson, V., Iqbal, M. P., Riach, I., and Waddell, S. 1995. *Ty1-copia* group retrotransposon sequences in amphibia and reptilia. Mol. Gen. Genet. 246:65-71.

Flavell, A. J., and Smith, D. B. 1992. A *Ty1-copia* group retrotransposon sequence in a vertebrate. Mol. Gen. Genet. 233:322-326.

Flavell, A. J., Smith, D. B., and Kumar, A. 1992b. Extreme heterogeneity of *Ty1-copia* group retrotransposons in plants. Mol. Gen. Genet. 231: 233-242.

Garfinkel, D. J. 1992. Retroelements in microorganisms. Pages 107-158 in: The Retroviridae. Vol. 1. J. A. Levy, ed. Plenum Press, New York.

Goodwin, S. B., Drenth, A., and Fry, W. E. 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Curr. Genet. 22:107-115.

Julien, J., Poirier-Hamon, S., and Brygoo, Y. 1992. *Foret*1, a reverse transcriptase-like sequence in the filamentous fungus *Fusarium oxysporum*. Nucleic Acids Res. 20:3933-3937.

Kohlstaedt, L. A., Wang, J., Rice, P. A., Friedman, J. M., and Steitz, T. A. 1993. The structure of HIV-1 reverse transcriptase. Pages 223-249 in: Reverse Transcriptase. A. M. Skalka and S. P. Goff, eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Margulis, L., Corliss, J. O., Melkonian, M., and Chapman, D. J., eds. 1989. Handbook of Protoctista. Jones and Bartlett, Boston.

Oliver, R. 1992. Transposons in filamentous fungi. Pages 3-11 in: Molecular Biology of Filamentous Fungi: Proc. EMBO-Worksh., Berlin, Aug. 24-28, 1991. U. Stahl and P. Tudzynski, eds. Weinheim, New York.

Rothnie, H. M., McCurrach, K. J., Glover, L. A., and Hardman, N. 1991. Retrotransposon-like nature of Tp1 elements: Implications for the organisation of highly repetitive, hypermethylated DNA in the genome of *Physarum polycephalum*. Nucleic Acids. Res. 19:279-286.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Tooley, P. W., Fry, W. E., and Villarreal Gonzalez, M. J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. J. Hered. 76:431-435.

Voytas, D. F., Cummings, M. P., Konieczny, A., Ausubel, F. M., and Rodermel, S. R. 1992. *copia*-like retrotransposons are ubiquitous among plants. Proc. Natl. Acad. Sci. USA 89:7124-7128.

Xiong, Y., and Eickbush, T. H. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J. 9:3353-3362.

Xu, D.-Y, Huang, H., and Wang, C.-P. 1982. Polyacrylamide gel disc electrophoresis of proteins from species of *Phytophthora*. Acta Mycol. Sin. 1:40-47.