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The Detection of Nonhybrid, Trisomic, and Triploid Offspring in Sexual Progeny of a Mating of *Phytophthora infestans*

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Carter, D. A., Buck, K. W., Archer, S. A., Van der Lee, T., Shattock, R. C., Shaw, D. S. 1999. The Detection of Nonhybrid, Trisomic, and Triploid Offspring in Sexual Progeny of a Mating of *Phytophthora infestans. Fungal Genetics and Biology* 26, 198–208. Eighty single-oospore offspring of *Phytophthora infestans* from a mating of isolates, which had previously been analyzed for segregation of avirulence/virulence, were assessed for the inheritance of 20 RFLP markers. Three offspring were triploid; they inherited three alleles at all loci where this could be detected and when heterozygous, showed unequal intensities of hybridization with most probes. Twenty-four offspring were trisomic, as each had three doses of one or a few markers, evident from their inheritance of three alleles or from unequal hybridization to one probe. Coinheritance of the extra allele(s) and mitochondrial haplotype in the majority of trisomic offspring suggested that meiosis in oogonia was more aberrant than in antheridia. Linkage analysis was performed on 50 offspring, which were assumed to be euploid; six small linkage groups were detected and several avirulence loci were found to be linked. The origins of aberrant offspring are discussed.

Index Descriptors: Phytophthora infestans; RFLP; linkage; avirulence; mitochondrial DNA; triploidy; trisomy; aneuploidy; Oomycetes.

Patterns of inheritance of genetic markers in chromistan fungi in the phylum Oomycota (Oomycetes) are normally those expected in sexual progeny from diploid parents. However, inheritance of some markers in many species is nonMendelian (reviewed in Judelson, 1996); the origins of these aberrations are usually unknown.

In *Phytophthora infestans,* codominant allozyme markers (Shattock *et al.,* 1987) and dominant RFLP (Goodwin *et al.,* 1992) and AFLP markers (Van der Lee *et al.,* 1997) show Mendelian inheritance, but mating type and closely linked molecular markers show aberrant segregation (Shaw and Shattock, 1991; Judelson *et al.,* 1995). Inheritance of avirulence to potato suggests that single dominant genes for avirulence match many of the single dominant genes for resistance (R-genes), but that avirulence to other R-genes might not be monogenic or dominant or that aberrations in the transmission of genes are responsible for unexpected results (Al-Kherb *et al.,* 1995; Spielman *et al.,* 1990). Here we examine the transmission of genes in progeny of a mating previously analyzed for the inheritance of avirulence to six R-genes; we use 20 codominant, single-locus RFLP markers, some detecting three or all four alleles at a single locus.

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MATERIALS AND METHODS

Parents and Offspring of Mating Ca65 × 550

The sexual progeny had already been established from a mating of an A1 isolate, Ca65, from tomato in southern California and an A2 isolate, 550, from a wild *Solanum* sp. in central Mexico and had been scored for the inheritance of avirulence/virulence. Single-oospore cultures had been established using Novozyme 234 to destroy viable sporangia and hyphal fragments. Oospores with single or multiple germ tubes were identified and isolated (Al-Kherb *et al.*, 1995). These isolates appeared to be suitable as parents for genetic mapping, as they differed at six avirulence loci and at some of the RFLP loci that were analyzed previously (Carter *et al.*, 1991). Genotypes of the parents at the glucosephosphate isomerase locus, *Gpi-1*, their mitochondrial haplotype, and their avirulence phenotype on differentials of potato, each carrying a different R-gene, are shown in Table 1.

We selected a subset of the progeny analyzed by Al-Kherb *et al.* (1995) to exclude offspring showing anomalous inheritance of RFLP markers. Inheritance of avirulence/virulence among these offspring is shown in Table 2 (adapted from Al-Kherb *et al.*, 1995).

DNA Extraction

The mycelium of each single-oospore isolate was grown in static liquid culture of pea-water broth (prepared by autoclaving 250 g frozen peas in 1L H_2O). A petri dish culture containing 25 ml of medium was inoculated with several mycelial plugs and incubated for 10–14 days at 18°C. The mycelium from a single plate (2–3 g) was washed, blotted dry, ground to a fine powder in liquid nitrogen, and extracted essentially as in Kolar *et al.* (1988) using a buffer containing *p*-aminosalicylic acid and triisonaphthalenesulphonic acid. After a treatment with phenol–chloroform–isoamyl alcohol, the DNA was purified by CsCl centrifugation, precipitated in sodium acetate–isopropanol, and spooled onto a plastic pipette tip. Final precipitation from TE buffer was in sodium acetate–ethanol. Spooled DNA was rinsed in 70% ethanol and dissolved in distilled water.

TABLE 1
Genetic Characteristics of Parental Strains

Parental strain	Genetic characteristics			
	Mating type[a]	Virulence phenotype[b]	mtDNA	GPI-1[c]
Ca65	A1	1, 4, 11	IIb	100/100
550	A2	1, 2, 3, 4, 5, 7, 10, 11	Ia	86/86

[a] Determined by pairing with a known A1 mating type and with a known A2 mating type on rye agar; oospores formed at the interface between the strains of different mating type within 1 week.

[b] Determined on detached leaflets of potato differentials; Ca65 is virulent on R1, R4, and R11 and avirulent on R2, R3, R5, R7, R8, and R10.

[c] 100 and 86 are alleles at the allozyme locus glucosephosphate isomerase 1 (Shattock *et al.*, 1987).

TABLE 2
Inheritance of Avirulence in Progeny from Ca65 × 550 Cross[a]

	Potato differentials					
	R2	R3	R5	R7	R8	R10
Parent genotypes: Ca65/550	−/+	−/+	−/+	−/+	−/−	−/+
No. progeny avirulent (−)	21	18	17	18	24	8
No. progeny virulent (+)	18	22	21	21	14	15
Expected ratios	1:1	1:1	1:1	1:1	3:1	1:1
χ^2 (−:+)	0.2	0.4	0.42	0.23	2.84	2.31
Probability	>0.6	>0.6	>0.6	>0.6	>0.05	>0.1

[a] Data determined by Al-Kherb *et al.* (1995). Only offspring inheriting one allele from each parent at all RFLP loci are included.

DNA Cloning

Probes pIN2, pIN11, and pIN29, derived from genomic DNA of *P. infestans*, have already been described (Carter *et al.*, 1991). pSTA99 was obtained from the University of St. Andrews (Unkles, unpublished). The remaining clones were constructed by fully digesting nuclear DNA (nDNA) of isolate 550 with *Hin*dIII and separating fragments by agarose–gel electrophoresis; fragments of approximately 0.5 kb were electrophoresed onto DEAE ion exchange paper (Maniatis *et al.*, 1982). The DNA was then ligated into *Hin*dIII-digested pUC19 (Cobianchi and Wilson, 1987) and used to transform *Escherichia coli* strain DH5a by electroporation using a Gene Pulser (Bio-Rad) and following the instructions of the manufacturer. DNA was prepared from recombinant clones by the rapid boiling technique (Maniatis *et al.*, 1982). Plasmids were digested with *Hin*dIII and electrophoresed to ensure that they carried a single insert of DNA. DNA was transferred from the agarose gels to Hybond N^+ membranes following the

SSPE method given in the manufacturer's recommendations (Amersham International) and membranes were then hybridized with radiolabeled genomic DNA. Only those clones which produced very faint hybridization signals were selected for subsequent testing to find single-copy and heterozygous RFLPs.

Restriction Enzyme Digestion, Southern Transfer, and Hybridization

Restriction enzymes from different sources were used according to the manufacturers' recommendations. Generally, 5–10 µg nDNA was restricted (2 U/µg) for 2–5 h. Electrophoresis was carried out in horizontal 0.7% agarose gels in TBE buffer for 16 h at 5 V/cm; 3 µg of DNA was loaded per lane. Digested DNA in agarose gels was transferred to Hybond N^+ membranes using the manufacturer's SSCP method (Amersham International) and the blot was fixed by UV crosslinking. Probes were radiolabeled by random priming of the insert DNA following its isolation in Nusieve GTG agarose (FMC BioProducts) (Feinberg and Vogelstein, 1983). Hybridization and stripping of filters was performed following the Hybond N^+ protocols. Complete digestion of the DNA was confirmed by hybridizing blots with the single-copy probe, pIN2 (Carter *et al.*, 1991). The intensity of hybridizing bands was assessed visually and aberrant hybridizations were recorded if allelic bands present in a single lane showed marked differences in intensity.

Assessment of mtDNA Haplotype

mtDNA haplotype was assessed on gels containing DNA digested with *Msp*I. As the mitochondrial genome contains a higher percentage of AT than genomic DNA of *P. infestans*, it is cut less frequently with this enzyme (recognition site CCGG). The larger mtDNA bands can therefore be seen above the bulk of digested DNA (Carter *et al.*, 1990). It was possible to assess mtDNA haplotypes Ia (550) and IIb (Ca65) using these bands. Results were confirmed by hybridizing DNA digested with *Bgl*II with the mtDNA probe, pSTA99.

Linkage Analysis

Linkage analysis was performed using JOINMAP version 1.4 (CPRO, Wageningen, The Netherlands) (Stam, 1993). Data were entered for each locus according to whether it segregated in the Ca65 or 550 parent, and separate maps were constructed for each parent using LOD score thresholds from 1.5 to 4.0. The individual maps were then integrated using markers that were present in both parents and which segregated in the progeny or markers detecting multiple alleles at a single locus. Figures were drawn based on those obtained from the companion programme DRAWMAP (Van Ooijen, 1994).

RESULTS

Hybridity of the Progeny

Of 123 offspring originally tested (Al-Kherb *et al.*, 1995), 120 were heterozygous, *Gpi-1 86/100,* at the glucosephosphate isomerase locus and were thus presumed to be hybrids, inheriting the *86* allele from the 550 parent and the *100* allele from the Ca65 parent. One offspring inherited only the *86* allele from the 550 and 2 offspring only the *100* allele from Ca65; only 1 of the latter, 222, was analyzed for other markers.

The hybridity of a subset of 80 progeny was further tested using RFLP marker pIN2; parents were homozygous for different alleles of this single-band marker. All except three offspring inherited one band from each parent (Fig. 1); as expected, offspring 222 inherited only the band from parent Ca65. Other RFLP probes showed that 222 had a genotype identical to Ca65, suggesting failure of meiosis. Offspring 307, although heterozygous when tested for *Gpi-1*, was found to be identical to parent 550 for pIN2 and for all other discriminating RFLP markers. Offspring 272 inherited alleles of *Gpi-1* and of *L5-62* (identified by probe pIN5-62) from both parents, but alleles at pIN2 and all other discernible RFLP loci from parent 550. Some of these loci were heterozygous in 550 but homozygous in 272, which is suggestive of selfing. pIN2 also revealed some pronounced differences in hybridization intensity in the two inherited bands (e.g., 208, 364, and 265), indicating more than one copy of one of the alleles in some heterozygous offspring (Fig. 1).

Inheritance of Mitochondrial DNA

As expected, parent Ca65 showed the restriction fragment haplotype IIb and parent 550 the haplotype Ia (Fig. 2a). Thirty-three progeny inherited their mtDNA from Ca65 (type IIb) and the remaining 47 progeny from 550 (type Ia). The same pattern of inheritance was detected by the probe pSTA99, which hybridized to different *Bgl*II

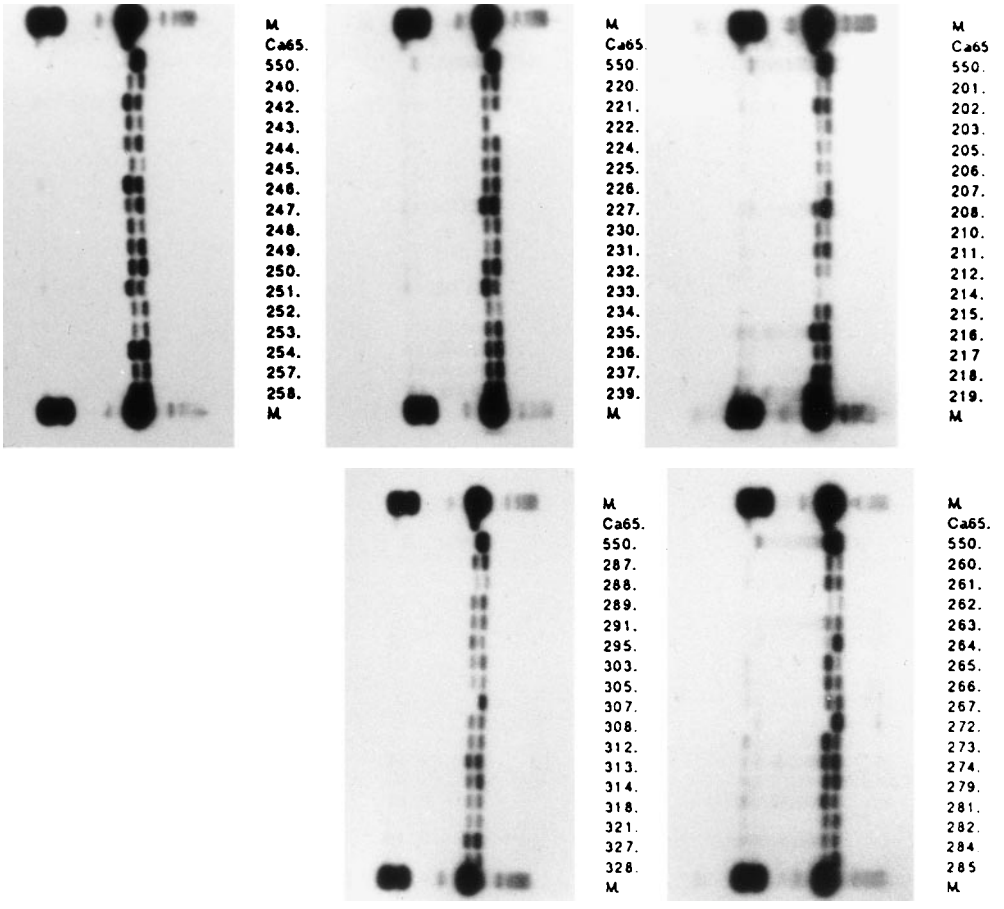

FIG. 1. Southern blot of *Msp*I-digested genomic DNA from Ca65, 550, and 80 offspring hybridized with pIN2.

fragments in blots of digested DNA from the two parents (Fig. 2b).

Inheritance of Nuclear RFLP Markers

Of 725 size-selected clones, the large majority contained DNA of a highly repetitive nature. Of those remaining, most were moderately repetitive and only 14 contained inserts that were suitable for mapping; i.e., they were single copy and revealed heterozygous loci in at least one parent. One clone, pIN5-216, contained insert DNA detecting moderately repetitive sequences that produced single-locus segregation. The majority of clones detected two alleles at their polymorphic locus, with one parent homozygous and the other heterozygous; all of the hybrid progeny should therefore be identical to one or the other parent.

Figure 3 shows representative autoradiographs of some of the progeny screened with three of the RFLP-detecting probes. As with pIN2, some probes hybridized unequally with the two bands present in some of the heterozygous progeny. For example, the upper band was more intense in offspring 261 and 265 hybridized with pIN11 (Fig. 3a), as was the lower band in offspring 247, 248, and 251 hybridized with pIN5-296 (Fig. 3b). The greater intensity in these cases suggests that an extra copy of one allele was present. Additional anomalies of a different kind can be seen in Fig. 3c, in which DNA digested with *Bgl*II was hybridized with pIN5-362. Offspring 295, 305, and 312 (and 201, not shown) possessed a hybridizing band that was not present in either parent or the rest of the progeny. These new bands hybridized less intensely than the band below them, which all of the progeny shared.

Four of the clones detected three alleles at a single locus, with one of these alleles present in both parents (Fig. 4). Most of the progeny inherited two of the three alleles as expected, but some examples of unequal hybridization intensities were also seen. In addition, pIN5-304 hybridized with all three alleles in eight of the progeny and

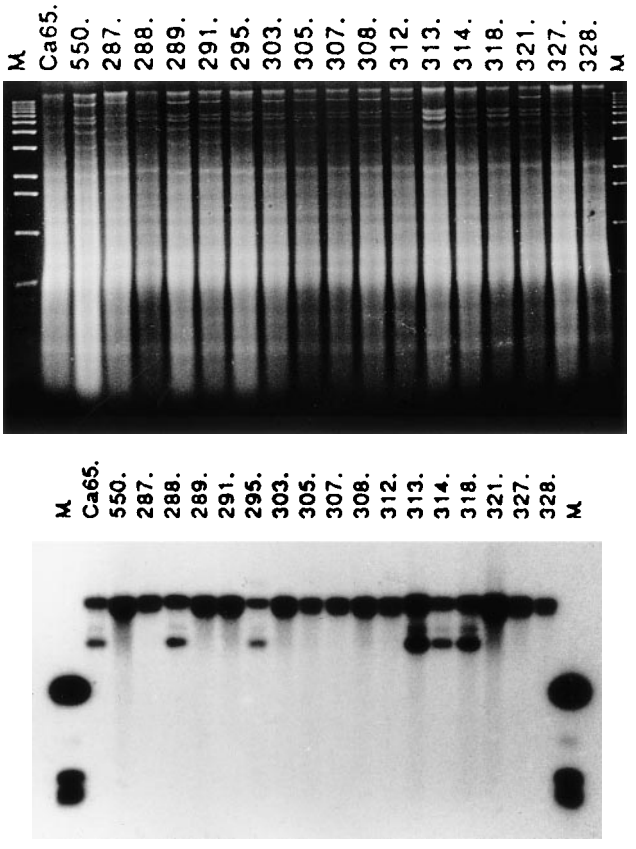

FIG. 2. (a) Agarose gel electrophoresis of DNA from Ca65, 550, and offspring 287–328 digested with *Msp*I. mtDNA bands are visible above the bulk of the digested nDNA. (b) Digested DNA from (a) hybridized with pSTA99, a plasmid probe containing a mtDNA insert.

pIN5-317 hybridized with three alleles in one offspring (Table 3).

One clone, pIN5-655, detected a locus with four different alleles. Ca65 and 550 were each heterozygous for two unique alleles, so that there were four possible genotypes in the progeny, none of which were the same as the parental genotype (Fig. 5). This is the most useful type of RFLP for genetic analysis of the progeny, as inheritance of each band from each parent is unambiguous. Two of the progeny showed unequal hybridization at this locus, and eight of the progeny carried three alleles (e.g., offspring 273 in Fig. 5). Of these, seven inherited two alleles from the Ca65 parent and one had the two alleles from 550. Where all three alleles were present together, hybridization was of equal intensity. All of the remaining offspring carried two alleles; none carried a single allele only, and none carried all four alleles.

Table 3 lists offspring having more than two alleles or unequal hybridization intensities and shows the parent from which the extra allele was inherited. This table also divides the progeny according to which parent contributed mtDNA. Eighteen progeny inherited one extra allele at one or more loci from Ca65; of these, 15 progeny coinherited mtDNA from Ca65. All 7 offspring inheriting an extra allele from parent 550 also inherited their mtDNA from 550.

The additional bands and unequal hybridization intensities did not appear to be due to cross contamination between isolates, as most progeny were abnormal at only one or two loci and were normal diploids at all others. However, offspring 208, 233, and 265 consistently carried more than two alleles at each locus at which they were heterozygous. Extra alleles were derived consistently from one of the parents. In offspring 265, several loci had one band that had more than twice the intensity of the other.

Linkage Analysis of Polymorphic Loci

Of the 80 offspring presumed from their *Gpi-1* genotype to be hybrids, 30 showed one or more of the above aberrations at RFLP loci and were omitted from the genetic analysis. This was considered necessary as it is possible that anomalies at other loci, in particular the avirulence loci, would go unnoticed and produce misleading results. Segregation ratios for the 19 RFLP and 6 avirulence loci, where one or both parents were heterozygous, tested by χ^2 were not significantly different ($P > 0.05$) from expected 1:1, 1:2:1, or 3:1.

The linkage groups obtained are shown diagrammatically in Fig. 6. Twelve loci did not show linkage. Logarithm of the Odds (LOD) scores shown alongside each linked pair express the Log_{10} of the ratio of the probability that the two loci are linked over the probability that the two are not linked, with a given recombination value. A LOD score can be seen as a measure of linkage information in the data. For linkage in human datasets, a LOD score threshold of 3 is generally set to establish proof of linkage between two loci. Values of 1 to 2 are considered 'interesting' and 2 to 3 'suggestive' of linkage (Botstein *et al.,* 1980). Tentative linkage between pair L5-216 and L5-725 and pair Avr-5 and Avr-8 is also shown on Fig. 6. These have LOD scores below the threshold of 3, but are sufficiently high to be considered interesting. These may reflect loose linkage, which may be confirmed by the analysis of more data.

DISCUSSION

Oomycetous fungi are more closely related to chromistan algae than to true fungi (e.g., Hawksworth *et al.,* 1995)

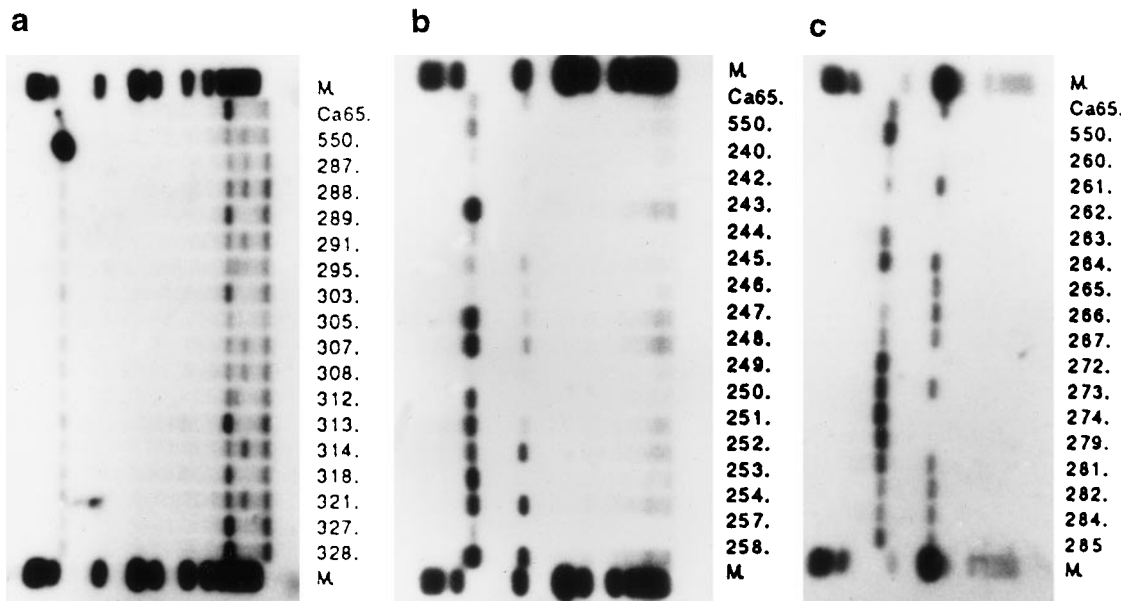

FIG. 3. Representative Southern blots showing hybridization of probes to digested genomic DNA from Ca65, 550, and a selection of offspring. (a) *Msp*I-digested DNA hybridized with pIN11; unequal hybridization seen in offspring 261. (b) *Hin*dIII-digested DNA hybridized with pIN5-296; unequal hybridization seen in offspring 247, 248, and 25. (c) *Bgl*II-digested DNA hybridized with pIN5-362; offspring 295, 305, and 312 possess a hybridizing band that is not present in either parent.

and have a diploid life cycle. In general, their inheritance is Mendelian. However, many examples of nonMendelian segregation of both classical (Shaw, 1983) and molecular markers (Judelson, 1996) have been reported.

We have mated Ca65, a tomato isolate from California, and 550, an isolate from *Solanum* sp. from central Mexico differing at 20 RFLP loci. Previous work had established that progeny segregated for avirulence/virulence to six

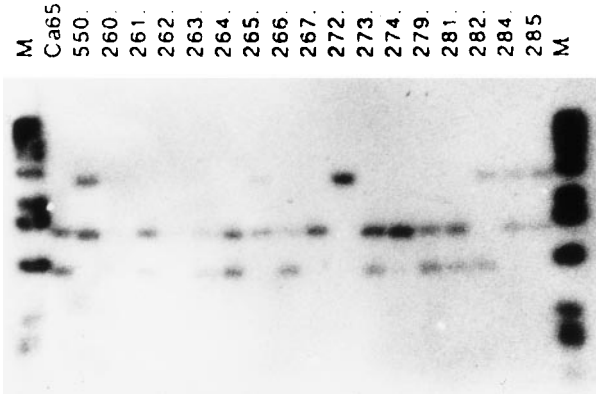

FIG. 4. Southern blot of *Msp*I-digested DNA from Ca65, 550, and offspring 260–285 hybridized with pIN5-317.

R-genes of potato (Al-Kherb *et al.*, 1995). Mitochondrial DNA haplotypes Ia and IIb of the parents were inherited uniparentally as expected (Whittaker *et al.*, 1994). As the mtDNA of dioecious *Pythium sylvaticum* is transmitted to the progeny only by the female parent (Martin, 1989), it has been assumed here that mitochondrial DNA (mtDNA) in bisexual *P. infestans* is transmitted through oogonia and not through antheridia. In this cross, 59% of the hybrid single-oospore progeny inherited mtDNA from parent 550, indicating that more of the oogonia giving rise to the progeny established here were formed by parent 550. This is in contrast to the findings of Judelson (1997) that Ca65 provided only oogonia in pairings with diverse A2 isolates; 550, although a stronger female than other diverse A2 isolates, did form a small proportion of antheridia with diverse A1 isolates. Perhaps rare oospores formed from oogonia of 550 had a much higher viability than those from oogonia of Ca65.

Linkage of Markers in Diploid Hybrid Offspring

From a progeny of 80 offspring, 50 showed expected diploid inheritance of 20 RFLP loci; these were analyzed for linkage of RFLP, avirulence, and mating-type markers.

TABLE 3
Inheritance of mtDNA in Offspring Showing Three Alleles or Unequal Hybridization Intensities

Progeny inheritance	Loci showing 3 alleles	Loci showing unequal hybridization of allelic bands
Extra alleles from Ca65; mtDNA from Ca65		
202		L5-62
207	L5-304	
212	L5-304	
234		L5-13
242	L5-304, L5-655	L29, L5-6
243	L5-304, L5-655	
245	L5-655	
246	L5-655	L5-13
248	L5-304, L5-655	L5-599
251	L5-317, L5-655	L5-599
260		L5-599
261	L5-655	L11
273	L5-655	
281	L5-304	
295	L5-304	
Extra alleles from 550; mtDNA from 550		
201		L5-6
208		L2, L5-6, L5-62, L5-216, L5-304, L5-362, L5-655
239		L5-216
244	L5-655	L5-6
264		L2, L5-216
305		L5-6
312		L5-6
Extra alleles from Ca65; mtDNA from 550		
233		L29, L5-62, L5-65, L5-216, L5-317, L5-655
265		L2, L11, L29, L5-174, L5-216, L5-304, L5-317, L5-599, L5-655
285	L5-304, L5-655	

Linkage analysis placed 15 loci into six small linkage groups, with the remaining 12 loci remaining unlinked (Fig. 6). This high proportion of unlinked loci probably reflects the large size of the *P. infestans* genome, which was estimated to be around 1200 cM (Van der Lee *et al.*, 1997) and has a high percentage of moderately and highly repetitive DNA (Carter *et al.*, 1991). No linkage was detected between any RFLP locus and the mating-type locus; similarly, avirulence loci were not linked to RFLPs. The linkage of *Avr3* and *Avr7* detected here using only the 50 normal offspring was also detected by Al-Kherb *et al.* (1995) using the total progeny. Analysis of the total progeny failed to detect linkage of *Avr5* and *Avr8* (Al-Kherb *et al.*, 1995), but exclusion of abnormal

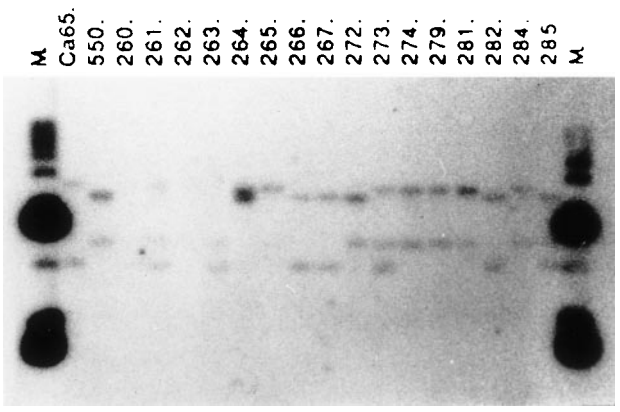

FIG. 5. Southern blot of *Msp*I-digested DNA from Ca65, 550, and offspring 260–285 hybridized with pIN5-655. Note presence of three alleles in offspring 261, 273, and 285.

offspring in the present analysis resulted in linkage of these loci. Some evidence of linkage between *Avr2* and *Avr4* was reported by Spielman *et al.*, (1990) and between *Avr7* and *Avr11*, and *Avr3* and *Avr11*, by Al-Kherb *et al.*, (1995) from crosses using other parents. As molecular markers were not used, aberrant offspring could not be detected in these progeny.

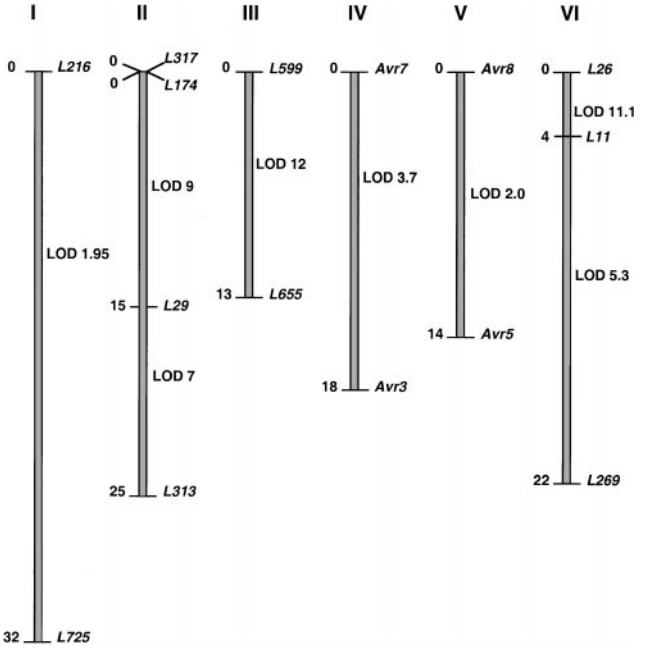

FIG. 6. Diagrammatic representation of linkage detected by JOINMAP version 1.4. Numbers shown beside each locus express distance between loci in CentiMorgans. Tentative linkage of markers is indicated. The following loci were unlinked: *Avr2, Avr10, mating type, L5-6, L5-13, L5-61, L5-62, L5-65, L5-199, L5-304, L5-362,* and *MT99.*

A recent, more intensive mapping in a progeny of *P. infestans* from parents isolated in The Netherlands using 183 segregating AFLP markers and 7 RFLP markers (detected with probes RG57 and pPi122) resulted in 10 major linkage groups (Van der Lee *et al.,* 1997). Markers showed Mendelian inheritance, except that of mating type and those heterozygous in the A1 parent and linked to mating type. The molecular markers were mostly dominant and thus did not allow the detection of aneuploid progeny. Marker saturation of the map may detect additional linkage groups but may also link one or more pairs of some linkage groups. The 10 major linkage groups detected so far are consistent with the cytological data of Sansome and Brasier (1973) (n = 8–10) and Whittaker *et al.* (1991) (n = 8–12). Analysis of 53 RFLP loci in two related matings of the lettuce downy mildew fungus, *Bremia lactucae,* detected 13 small linkage groups (Hulbert *et al.,* 1988). The absence of abnormal offspring with additional alleles in these crosses might be related to the obligate sexual stage in the life cycle of this biotrophic pathogen.

Nonhybrid Progeny

In matings of *P. infestans,* homozygous isoenzyme markers (mainly *Gpi-1*) have been employed to distinguish the majority of offspring, which are heterozygous and thus hybrid, from those that inherit an allele from one parent only; the latter have been generally thought to be nonhybrid offspring produced by selfing (automixis) of one parent induced by pheromones from the other parent (e.g., Shattock *et al.,* 1987). However, evidence of selfing, such as segregation of a heterozygote, has usually been lacking. Offspring 222, a presumptive self of Ca65 according to its *Gpi-1* marker, proved to be identical to Ca65 at all 19 RFLP loci, even at those heterozygous in Ca65, and also possessed type IIb mtDNA. This offspring was thus not a product of automixis, but could have resulted from one or more unreduced and unfertilized parental nuclei. Offspring 307 was heterozygous at the *Gpi-1* locus but, when tested some months later, was identical to parent 550 for the 20 RFLP loci and mtDNA haplotype. Survival of an unreduced parental nucleus is indicated but the *Gpi-1* allele from parent Ca65 suggests that fertilization occurred. Several interpretations are possible, e.g., perhaps the oospore contained one hybrid and one unreduced nucleus and formed a heterokaryotic colony that later lost the hybrid nucleus; alternatively, the unreduced nucleus was fertilized and the triploid product preferentially lost chromosomes from parent Ca65 during growth. Another *Gpi-1* heterozygote, 272, also inherited most RFLP alleles and mtDNA from parent 550; in this case, some loci, heterozygous in 550, were homozygous in 272, as expected if both gametic nuclei were from 550. However, the presence of an allele of *Gpi-1* and also of *L5-62* from parent Ca65 suggests the involvement of a gametic nucleus of that parent. One possibility is failure of meiosis II in a 550 nucleus, karyogamy with a gametic nucleus of Ca65, and loss of most chromosomes of Ca65 during mitosis.

Förster and Coffey (1990) analyzed progeny of two crosses of *P. nicotianae* (syn. *P. parasitica*) in which parents differed for several homozygous RFLP markers. Most offspring were heterozygous at all four or five RFLP loci, respectively, and were thus hybrid, but a few inherited the allele from one parent only at all loci; these latter could have been parentals or selfs or even partial hybrids. Almost half of the progeny were some sort of partial hybrid in that they inherited most markers from both parents but one or two from one parent only. From our results and those of Förster and Coffey (1990), it is clear that lack of transmission of a single marker from one parent cannot be used to distinguish among offspring which are parental, selfs, or partial hybrids.

Triploid Offspring

In three offspring, there was an unequal intensity of hybridization at many loci, indicating transmission of a double dose of one allele from one of the parents. In 208, both mtDNA and the extra alleles were from 550, whereas in 233 and 265, mtDNA was from 550 and extra alleles were from Ca65 (Table 3). In all three offspring, a triple fusion of gametic nuclei or involvement of an unreduced gamete can be ruled out as, in every case, the two alleles from one parent were identical. This is confirmed by probe pIN5-655, which detected only two of the four possible alleles but did show unequal hybridization in all three offspring. This argues for the duplication of one gametic nucleus before fusion or failure of meiosis II, but the latter would yield a gametic nucleus heterozygous for distal markers. Offspring 265 may be more complex as the intensity of hybridization at some loci was distinctly more than twice that of the band from the other parent (Fig. 1).

Nontriploid Offspring with Extra Alleles

Twenty-four of the progeny carried three alleles (indicated by three distinct allelic bands or by heterozygotes with one band approximately twice as intense as the other)

at one or several loci but not at others. Duplication could have arisen at meiosis by unequal crossing over, possibly mediated by repetitive DNA (Kistler *et al.,* 1995). This would have generated two identical copies of one allele, but whereas this could explain more intense hybridization it cannot explain the presence of three different alleles, with two of these coming from one parent. It is therefore likely that these offspring are trisomic, with the extra chromosome resulting from nondisjunction at meiosis or from repeated mitotic nondisjunction of a triploid. Offspring with two (or several) triplicated loci could be trisomic if the loci are linked, or $2n + 2$ (3 etc.) if unlinked. Analysis did not detect linkage of any of these loci and thus these hypotheses cannot be distinguished by this means.

It is clear from Table 3 that 15 of the 16 putative trisomic progeny that inherit a second allele from Ca65 also inherit its mtDNA (the exception is 285 with mtDNA from 550). Similarly, all six trisomics inheriting the extra allele(s) from 550 also inherit its mtDNA. Assuming maternal inheritance of mtDNA, this suggests that aberrations (e.g., disomic gametes) may be generated more often in oogonia than in antheridia of both parents. Meiosis would seem to be more accurate in 550 than in Ca65. Perhaps selection in a predominantly sexual population in central Mexico (550) has maintained accurate division at meiosis in the 550 lineage; in populations thought to be predominantly asexual from California (Ca65) (Vartanian and Endo, 1985), such selection may have been relaxed.

Trisomy was invoked to explain the origin of the abnormal, partially hybrid offspring of *P. nicotianae* from two matings mentioned above (Förster and Coffey, 1990). For example, in one mating, more than half of the hybrid progeny did not inherit an allele from one parent at one or two of the five RFLP loci determined. A trisomic offspring could have lost one of the three homologous chromosomes by mitotic nondisjunction to leave it with two homologues from one parent or the offspring could initially have been triploid and lost chromosomes randomly during mitosis. In this same mating, two hybrid offspring showed unequal hybridization of the alleles detected by two RFLP probes, suggesting that these offspring were still trisomic.

There is increasing evidence that trisomics are not uncommon, even in field populations of oomycetous fungi, including *Phytophthora.* A widespread clone of *P. infestans* in the United States (US-8) was found to be five-banded for glucosephosphate isomerase, suggesting that it has one or more extra chromosomes (Goodwin *et al.,* 1995). Cytological evidence of an extra chromosome showed that a self-fertile isolate of *B. lactucae* from the field was a tertiary trisomic (Michelmore and Sansome, 1982). Similar evidence of tertiary trisomy was found in a self-fertile, single-oospore culture of *P. drechsleri* (Mortimer *et al.,* 1977; Sansome, 1980). Studies of DNA content (C-values) of somatic nuclei in some field strains of *P. infestans* revealed not only 3C and 4C (triploids and tetraploids) in some strains but also intermediate values, suggesting a range of aneuploids in others (reviewed by Shaw and Shattock, 1991). This tolerance of ploidy variation may be related to the absence or rarity of sexual reproduction in these populations.

Recent electrophoretic karyotype analysis of isolates of heterothallic *Py. sylvaticum* from a single field showed high levels of length polymorphism of chromosomal-sized DNA molecules (Martin, 1995). Karyotypes of F1 progeny from highly polymorphic parents were highly diverse. Southern blots of gels of parents and progeny were probed with cDNA clones and RAPD-derived probes; 80% of the chromosomes of offspring were nonparental in length and sequence groupings. It is thus not surprising that the inheritance of 6 of 12 RAPD markers was aberrant (e.g., a sequence amplified in one parent only was inherited by 45 offspring but not by another 3). In *B. lactucae,* variable numbers of small, possibly dispensable, chromosome-sized DNA molecules showing nonMendelian transmission have been detected (Francis and Michelmore, 1993). Although most intact chromosomal DNA molecules from *P. infestans* fail to migrate on pulsed field gel electrophoresis (Tooley and Carras, 1992), blotting of gels of 550 with pSTA99 (a mtDNA probe) hybridized to rapidly migrating mtDNA but also to a single band of ca. 2 Mb—perhaps from an unusually small chromosome including some mitochondrial sequences (Carter, 1991).

Even in the homothallic species, *P. sojae,* inheritance of molecular markers in F2 hybrids of outcrossed parents was frequently anomalous. Whisson *et al.* (1995) found that about ⅕ of their RFLP and RAPD markers which segregated in the expected 3:1 ratio in one F2 progeny showed aberrant segregation in another mating; about half of these markers were clustered in two segments of their linkage map. Tyler *et al.* (1995) also detected deviant segregations in their outcrosses of *P. sojae.* In one mating with 25 markers, two linked codominant RFLPs, expected to segregate 1:2:1, showed an excess of heterozygotes; Southern blots showed at least three different classes of heterozygote with unequal intensities of hybridization. It was concluded that the F1 was trisomic or carried a translocation. In a second mating, six of eight markers showed

anomalous F2 ratios; e.g., the recessive homozygote predominated.

The high frequency of aberrant offspring in this study may be typical of matings between field isolates of *P. infestans* or may be a product of mating between highly polymorphic parents, separated by a large genetic distance. Codominant RFLP markers, particularly those able to detect multiple alleles, are ideal for analyses of this kind and should be exploited to determine the relative normality of matings between various parents, including those from sexual and nonsexual populations.

REFERENCES

Al-Kherb, S. M., Fininsa, C., Shattock, R. C., and Shaw, D. S. 1995. The inheritance of virulence of *Phytophthora infestans* to potato. *Plant Pathol.* **44:** 552–562.

Botstein, D., White, R., Skolnick, M., and Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **32:** 314–331.

Carter, D. A. 1991. *DNA Polymorphisms as Genetic Markers in* Phytophthora infestans. PhD thesis, Imperial College, Univ. of London.

Carter, D. A., Archer, S. A., Buck, K. W., Shaw, D. S., and Shattock, R. C. 1990. Restriction fragment polymorphisms of mitochondrial DNA of *Phytophthora infestans. Mycol. Res.* **94:** 1123–1128.

Carter, D. A., Archer, S. A., Buck, K. W., Shaw, D. S., and Shattock, R. C. 1991. DNA polymorphisms in *Phytophthora infestans:* the UK experience. In *Phytophthora* (J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, Eds.), pp. 272–295. Cambridge Univ. Press, Cambridge, UK.

Cobianchi, G. F., and Wilson, S. H. 1987. Enzymes for modifying and labelling DNA and RNA. In *Guide to Molecular Cloning Techniques* (S. L. Berger and A. R. Kimmel, Eds.), pp. 94–110. Academic Press, New York.

Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **71:** 6–13.

Förster, H., and Coffey, M. D. 1990. Mating behavior of *Phytophthora parasitica:* Evidence for sexual recombination in oospores using DNA restriction fragment length polymorphisms as genetic markers. *Exp. Mycol.* **14:** 351–359.

Francis, D. M., and Michelmore, R. W. 1993. Two classes of chromosome-sized molecules are present in *Bremia lactucae. Exp. Mycol.* **17:** 284–300.

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

Goodwin, S. B., Schneider, R. E., and Fry, W. E. 1995. Use of cellulose–acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans. Plant Disease* **79:** 1181–1185.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C., and Pegler, D. N. 1995. *Ainsworth & Bisby's Dictionary of the Fungi.* 8th ed. CAB International, Wallingford.

Hulbert, S. H., Ilott, T. W., Legg, E. J., Lincoln, S. E., Lander, E. S., and Michelmore, R. W. 1988. Genetic analysis of the fungus *Bremia lactucae,* using restriction fragment length polymorphisms. *Genetics* **120:** 947–958.

Judelson, H. S. 1996. Recent advances in the genetics of oomycete plant pathogens. *Mol. Plant–Microbe Interact.* **9:** 443–449.

Judelson, H. S. 1997. Expression and inheritance of sexual preference and selfing potential in *Phytophthora infestans. Fungal Genet. Biol.* **21:** 188–197.

Judelson, H. S., Spielman, L. J., and Shattock, R. C. 1995. Genetic mapping and non-Mendelian segregation of mating type loci in the Oomycete, *Phytophthora infestans. Genetics* **141:** 503–512.

Kistler, C. H., Benny, U., Bochm, E. W. A., and Katan, T. 1995. Genetic duplication in *Fusarium oxysporum. Curr. Genet.* **28:** 173–176.

Kolar, M., Punt, P. J., Cees, A. M., van den Hondel, J. J., and Schwab, H. 1988. Transformation of *Penicillium chrysogenum* using dominant selection markers and expression of an *Escherichia coli lacZ* fusion gene. *Gene* **62:** 127–134.

Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Martin, F. 1995. Meiotic instability of *Pythium sylvaticum* as demonstrated by inheritance of nuclear markers and karyotype analysis. *Genetics* **139:** 1233–1246.

Martin, F. N. 1989. Maternal inheritance of mitochondrial DNA in sexual crosses of *Pythium sylvaticum. Curr. Genet.* **16:** 373–374.

Michelmore, R. W., and Sansome, E. 1982. The cytology of heterothallism and secondary homothallism in *Bremia lactucae. Trans. Br. Mycol. Soc.* **79:** 291–297.

Mortimer, A. M., Shaw, D. S., and Sansome, E. R. 1977. Genetic studies of secondary homothallism in *Phytophthora drechsleri. Arch. Microbiol.* **111:** 255–259.

Sansome, E. R. 1980. Reciprocal transloction heterozygosity in heterothallic species of *Phytophthora* and its significance. *Trans. Br. Mycol. Soc.* **74:** 175–185.

Sansome, S., and Brasier, C. M. 1973. Diploidy and chromosomal structural hybridity in *Phytophthora infestans. Nature* **241:** 344–345.

Shattock, R. C., Tooley, P. W., Sweigard, J., and Fry, W. E. 1987. Genetic studies of *Phytophthora infestans.* In *Genetics and Plant Pathogenesis* (P. R. Day and G. J. Jellis, Eds.), pp. 175–185. Blackwell Sci., Oxford.

Shaw, D. S. 1983. The Peronosporales—A fungal geneticist's nightmare. In *Zoosporic Plant Pathogens* (S. T. Buczacki, Ed.), pp. 85–122. Academic Press, London.

Shaw, D. S., and Shattock, R. C. 1991. Genetics of *Phytophthora infestans:* The Mendelian approach. In *Phytophthora* (J. A. Lucas, R. C., Shattock, D. S. Shaw, and L. R. Cooke, Eds.), pp. 218–230. Cambridge Univ. Press, Cambridge, UK.

Spielman, L. J., Sweigard, J. A., Shattock, R. C., and Fry, W. E. 1990. The genetics of *Phytophthora infestans:* Segregation of allozyme markers in F2 and backcross progeny and the inheritance of virulence against potato resistance genes R2 and R4 in F1 progeny. *Exp. Mycol.* **14:** 57–69.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. *Plant J.* **3:** 739–744.

Tooley, P. W., and Carras, M. M. 1992. Separation of chromosomes of *Phytophthora* species using CHEF gel electrophoresis. *Exp. Mycol.* **16:** 188–196.

Tyler, B. M., Förster, H., and Coffey, M. D. 1995. Inheritance of avirulence factors and restriction fragment length polymorphism markers in outcrosses of the oomycete *Phytophthora sojae*. *Mol. Plant–Microbe Interact.* **8:** 515–523.

Van der Lee, T., De Witte, I., Drenth, A., Alfonso, C., and Govers, F. 1997. AFLP linkage map of the Oomycete *Phytophthora infestans*. *Fungal Genet. Biol.* **21:** 278–291.

Van Ooijen, J. W. 1994. DrawMap: A computer program for drawing genetic linkage maps. *J. Heredity* **85:** 66.

Vartanian, V. G., and Endo, R. M. 1985. Overwintering hosts, compatability types, and races of *Phytophthora infestans* on tomato in southern California. *Plant Disease* **69:** 516–519.

Whisson, S. C., Drenth, A., Maclean, D. J., and Irwin, J. A. G. 1995. *Phytophthora sojae* avirulence genes, RAPD, and RFLP markers used to construct a detailed genetic linkage map. *Mol. Plant–Microbe Interact*. **8:** 988–995.

Whittaker, S. L., Assinder, S. J., and Shaw, D. S. 1994. Inheritance of mitochondrial DNA in *Phytophthora infestans*. *Mycol. Res.* **98:** 569–575.

Whittaker, S. L., Shattock, R. C., and Shaw, D. S. 1991. Variation in content of nuclei of *Phytophthora infestans* as measured by a microfluorimetric method using the fluorochrome DAPI. *Mycol. Res.* **95:** 602–610.