The Evolution of Races of *Phytophthora sojae* in Australia

A. Drenth, S. C. Whisson, D. J. Maclean, J. A. G. Irwin, N. R. Obst, and M. J. Ryley

First, second, third, and fourth authors: Cooperative Research Centre for Tropical Plant Pathology, Level 5 John Hines Building, The University of Queensland, Brisbane, 4072, Australia; fifth and sixth authors: Department of Primary Industries, P.O. Box 102, Toowoomba, Queensland 4350, Australia.

This work was partially funded by the Australian Research Council, the Grains Research and Development Corporation, and the Cooperative Research Centre for Tropical Plant Pathology.

We thank J. D. Paxton, C. R. Grau, and J. D. Heritage for supplying a number of isolates from the United States and Australia.

Accepted for publication 16 October 1995.

ABSTRACT

Drenth, A., Whisson, S. C., Maclean, D. J., Irwin, J. A. G., Obst, N. R., and Ryley, M. J. 1996. The evolution of races of *Phytophthora sojae* in Australia. Phytopathology 86:163-169.

Isolates of *Phytophthora sojae* collected between 1979 and 1993 from three soybean-growing regions in eastern Australia and the United States were characterized for virulence and restriction fragment length polymorphisms (RFLPs). A total of 99 isolates were characterized: 84 from Australia and 15 from the United States. In the Australian population of *P. sojae*, five races (1, 4, 13, 15, and X) were identified. Five RFLP probes were selected that detected 30 fragments, of which 7 were polymorphic, leading to the identification of three multilocus RFLP genotypes amongst the Australian isolates. One multilocus RFLP genotype occurred in over 95% of all samples collected throughout the soybean-growing regions of eastern Australia. The 15 isolates obtained from the United States represented 12 races; the five RFLP probes detected 31 fragments, of which 13 were polymorphic, corresponding to 12 multilocus RFLP genotypes. The low levels of genotypic diversity (2.5 to 14.3%) observed in Australian *P. sojae* populations compared to the high level (60%) observed in the U.S. population indicates that the Australian population was most likely established by a single introduction of the pathogen. All five races in Australia occurred in the same multilocus RFLP genotype, suggesting that new races in Australia evolve from a common genetic background through mutation.

Additional keywords: Oomycetes, outcrossing, *Phytophthora megasperma* f. sp. *glycinea*, population genetics, virulence.

The Oomycete *Phytophthora sojae* M.J. Kaufmann & J.W. Gerdemann (7) (synamorph *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D.C. Erwin) is a soilborne pathogen causing root and stem rot disease of soybean (22,23). *P. sojae* is diploid, homothallic (self-fertile), and host-specific to soybean. Oospores are readily produced in diseased soybean plant tissue and are considered the sole means by which the disease is carried forward from year to year (22). The geographic origin of *P. sojae* is unclear, and although its host, soybean, originated in southeastern Asia, some workers suggest it may be native to the United States (5,23).

Control of this disease has been achieved largely through breeding resistant cultivars (1,22). At present, 13 resistance genes (*Rps* genes) have been characterized in soybean (26). However, *P. sojae* has been able to overcome these resistance genes by generating new races (11,13,21). More than 37 races of *P. sojae* have been detected worldwide (5,13).

Phytophthora root rot of soybean was first recorded in Australia in 1979 (17) and quickly became an important problem in soybean production areas (19). Race 1 of *P. sojae* was first isolated in southeastern Queensland in 1979 (17). The following year race 15 also was identified in this region. Seasonal disease surveys conducted from 1979 to 1988 in southeastern Queensland and northern New South Wales (8,20) showed that 93% of all isolates were race 1, with the remainder from race 15. These proportions remained virtually unchanged between seasons until 1989. Race 4 was recorded for the first time in Australia in southern New South Wales near Forbes in 1990 (21). The appearance of race 4 is closely related to cultivation of cultivars Ridley and Lachlan, which are resistant to races 1 and 15 but susceptible to race 4 (21,25).

Despite the fact that *P. sojae* is a soilborne pathogen, the development and spread of new races occurs remarkably quickly. Several mechanisms can be hypothesized to explain the occurrence of new races of *P. sojae* in Australia, including mutations, genetic recombination (sexual and parasexual), the presence of an indigenous wild-type population, and the direct introduction of new races from overseas to Australia. To investigate the relative importance of these possible mechanisms, it is first necessary to study the genetic structure of the pathogen population in detail. Phenotypic and genotypic markers such as electrophoretic protein patterns and isozymes have been used with limited success to detect variation among isolates of *P. sojae* in Australia (9) and the United States (16). DNA-based markers, such as restriction fragment length polymorphisms (RFLPs), have been more successful in detecting genetic variation in *P. sojae* populations. The first application of DNA-based markers to *P. sojae* was by Förster et al. (4) who identified four groups among 24 isolates using mitochondrial DNA RFLPs.

In a pilot survey including five Australian and five North American isolates of *P. sojae*, genomic DNA probes, cDNA probes, and random amplified polymorphic DNA (RAPD) markers revealed a low level of genetic variation among the five Australian isolates (30; S. C. Whisson and E. C. Y. Liew, *unpublished data*). A higher level of genetic variation was observed among the five isolates from the United States. This study indicated that it was most likely that a single introduction of one or a limited number of genetically similar isolates of *P. sojae* to Australia had occurred (30). More recently, Förster et al. (5) observed a substantial level of genetic variation using a large set of low-copy RFLP

Corresponding author: A. Drenth; Fax: +61 733654771

Publication no. P-1995-1207-01R

probes among 48 isolates of *P. sojae* mainly collected in the United States. The observed relationships between RFLP groups led Förster et al. (5) to hypothesize that new races in the United States arose both by mutation and rare outcrosses among existing races.

Recent research demonstrating outcrossing within homothallic Oomycete species (2,5,6,27,28) has made it possible to initiate genetic analysis of this important group of plant pathogens. By coculturing genetically different strains of *P. sojae* and using RAPD markers to detect hybrid oospores among predominantly selfed progeny of the parental strains, Whisson et al. (27) demonstrated that a low level (<5%) of outcrossing can occur in vitro. The hybrids were subsequently used to generate an F_2 population in which segregation for virulence and molecular markers were analyzed. The demonstration of outcrossing in homothallic *Phytophthora* species in vitro immediately raises the possibility that outcrossing between different races may contribute to the appearance of new races of *P. sojae* in the field.

The possibility that the Australian population of *P. sojae* is derived from a single introduction has presented us with a unique opportunity to study the evolution of races in this pathogen. The aim of the current investigation was to use molecular (RFLP) and virulence markers to determine the population genetic structure of *P. sojae* in Australia. Comparisons were made with U.S. isolates to assist in identifying the source of the Australian population. RFLP markers that distinguish individual strains among the Australian population could subsequently be used to track the spread of clonal lines and provide information on mechanisms leading to the development of new races in Australia. Such information is vital to the successful implementation of durable disease management strategies in Australia.

MATERIALS AND METHODS

***P. sojae* isolates.** The 84 Australian *P. sojae* isolates included in this investigation were obtained as part of an ongoing Phytoph-

TABLE 1. List of Australian and U.S. isolates of *Phytophthora sojae* used

Strain	Year	Location	Location No.[a]	Cultivar	Race	RFLP[b] genotype
Southeastern Queensland						
UQ60	1979	Gatton	1	...	1	1
9933R15	1982	Southeastern Queensland	...	...	15	1
UQ15	1984	Gatton	1	...	1	1
UQ16	1984	Gatton	1	...	1	1
UQ17	1984	Gatton	1	...	1	1
UQ18	1984	Gatton	1	...	1	1
UQ20	1984	Gatton	1	...	1	1
UQ322	1991	Brookstead	2	Semstar	1	1
UQ237	1991	Brookstead	2	...	15	1
UQ324	1991	Brookstead	2	Semstar	15	1
UQ323	1991	Brookstead	2	...	15	1
UQ294	1991	Hermitage Research Station	3	Semstar	15	1
UQ310	1991	Hermitage Research Station	3	Semstar	1	1
UQ311	1991	Hermitage Research Station	3	Semstar	1	1
UQ312	1991	Hermitage Research Station	3	PK380	15	1
UQ313	1991	Hermitage Research Station	3	MACS13	...	1
UQ314	1991	Hermitage Research Station	3	Davis	15	1
UQ315	1991	Hermitage Research Station	3	IPB21-75	...	1
UQ317	1991	Hermitage Research Station	3	IPB27-75	15	1
UQ318	1991	Hermitage Research Station	3	A143H8	...	1
UQ319	1991	Hermitage Research Station	3	A143H8	4	1
UQ320	1991	Hermitage Research Station	3	IPB116-76	15	1
UQ321	1991	Hermitage Research Station	3	Dragon	1	1
UQ316	1993	Hermitage Research Station	3	ED74-260	15	1
UQ411	1993	Hermitage Research Station	3	Semstar	15	1
UQ412	1993	Hermitage Research Station	3	Rps3	15	1
UQ413	1993	Hermitage Research Station	3	Rps3	15	1
UQ414	1993	Hermitage Research Station	3	V.Line	13	1
UQ415	1993	Hermitage Research Station	3	Semstar	1	1
UQ416	1993	Hermitage Research Station	3	Semstar	1	1
Northern New South Wales						
UQ342	1992	Narrabri Research Station	4	Intrepid	1	1
UQ405	1992	Narrabri Research Station	4	EHP134	1	1
UQ407	1992	Narrabri Research Station	4	EHP128	1	1
UQ406	1993	Narrabri Research Station	4	EHP128	1	1
UQ408	1993	Narrabri Research Station	4	F6 Ruse	1	1
UQ409	1993	Narrabri Research Station	4	F6 Ruse	1	1
UQ410	1993	Narrabri Research Station	4	F6 Ruse	1	1
Southern New South Wales						
CIFR6	1983	Stanbridge	5	Chaffey	1	1
CIFR7	1983	Nartrangie	6	Farrer	1	1
CIFR10	1983	Bedgerebong	7	Farrer	1	3
CIFR11	1983	Forbes	8	Farrer	1	1
UQ246	1991	Coleambally	9	Bowyer	4	1
UQ302	1991	Coleambally	9	Bowyer	4	1
UQ303	1991	Coleambally	9	Ridley	4	1
UQ304	1991	Coleambally	9	Ridley	4	1
UQ305	1991	Coleambally	9	...	4	1

(continued on next page)

[a] Location numbers correspond to the map in Figure 1.
[b] Restriction fragment length polymorphism.

thora root rot survey from soybean fields at a number of locations in the three major soybean-growing regions (southeastern Queensland, northern New South Wales, and southern New South Wales) in eastern Australia (Table 1; Fig. 1). Fifteen isolates belonging to races 1, 4, 5, 7, 8, 9, 10, 13, 15, 21, 22, and 25 (race classification of Ward [26]) were from the United States. All isolates are stored cryogenically and form part of the *Phytophthora* culture collection of the Cooperative Research Centre for Tropical Plant Pathology at the University of Queensland, Brisbane.

Virulence/avirulence testing. Soybean cultivars Harosoy 63 (*Rps*1a), Sanga (*Rps*1b), WellsII (*Rps*1c), PI103091 (*Rps*1d), PI86972-1 (*Rps*3a), Harosoy 5272 (*Rps*5), Altona (*Rps*6), and Harosoy (*Rps*7) were used as differentials to evaluate the virulence spectrum of the *P. sojae* isolates. Virulence was tested using the hypocotyl inoculation technique described by Ryley et al. (21), using at least 10 plants of each of the above soybean cultivars for each isolate. Seedlings were assessed 4 days postinoculation and were rated as susceptible (hypocotyl collapsed) or resistant (no lesion). Isolates were classified as avirulent if at least 9 of 10 plants were resistant and virulent if at least 9 of 10 plants were killed. Cultures that killed between 10 and 90% of the plants tested were retested until the reaction could be classified as consistently virulent or avirulent. Unequivocal results were typically obtained on the second retesting for all isolates.

DNA extraction, enzyme digestion, and electrophoresis. Each isolate was maintained on clarified V8 agar (18). Mycelium was grown and harvested as described by Liew et al. (14). DNA was extracted from freeze-dried mycelium using a scaled up method of Yoon et al. (31). The restriction endonucleases used were *Xho*I and *Kpn*I (New England Biolabs, Beverley, MA). Restricted DNA (5 µg) was size fractionated on a 0.8% agarose gel and alkaline transferred onto Hybond N^+ membrane (Amersham, Australia).

Selection of RFLP probes and hybridization conditions. Selection of RFLP probes was based on a screening by Whisson et al. (29,30) of 10 random cDNA clones and 10 random genomic DNA clones in combination with 8 restriction endonucleases (each with

TABLE 1. *(continued from preceding page)*

Strain	Year	Location	Location No.[a]	Cultivar	Race	RFLP[b] genotype
UQ417	1991	Coleambally	9	NCW991	4	1
UQ298	1991	Condobolin	10	Lachlan	4	1
UQ299	1991	Condobolin	10	Ridley	4	1
UQ300	1991	Condobolin	10	VNC138	4	1
UQ308	1991	Deniliquin	11	Bowyer	4	1
UQ295	1991	Dubbo	12	Forrest	1	1
UQ238	1991	Finley	13	Ballow	X	1
UQ309	1991	Finley	13	Bowyer	1	1
UQ236	1991	Forbes	8	Lachlan	4	1
UQ243	1991	Forbes	8	Lachlan	4	1
UQ297	1991	Forbes	8	Lachlan	4	1
UQ245	1991	Hay	14	Stephens	1	1
UQ307	1991	Jerilderie	15	...	1	1
UQ235	1991	Leeton	16	...	1	1
UQ296	1991	Narromine	17	Forrest	1	2
UQ301	1991	Yanco Research Station	18	...	1	1
UQ326	1992	Coleambally	9	Lorna	4	1
UQ335	1992	Condobolin	10	88/3047	4	1
UQ336	1992	Condobolin	10	Ridley	4	1
UQ337	1992	Condobolin	10	WNC173	4	2
UQ339	1992	Condobolin	10	Stephens	4	1
UQ340	1992	Condobolin	10	WNC39	4	1
UQ341	1992	Condobolin	10	Lachlan	4	1
UQ328	1992	Jerilderie	15	Stephens	15	1
UQ329	1992	Jerilderie	15	...	4	1
UQ330	1992	Jerilderie	15	...	4	1
UQ331	1992	Jerilderie	15	...	4	1
UQ333	1992	Jerilderie	15	Stephens	4	1
UQ334	1992	Jerilderie	15	Bowyer	4	1
UQ418	1993	Condobolin	10	NCW178	4	1
UQ420	1993	Condobolin	10	V. Line	4	1
UQ421	1993	Condobolin	10	...	4	1
UQ422	1993	Condobolin	10	Lorna	4	1
UQ423	1993	Condobolin	10	...	1	1
UQ425	1993	Condobolin	10	Semstar	4	1
UQ404	1993	Finley	13	Stephens	15	1
UQ426	1993	Wirrinya	8	...	4	1
UQ427	1993	Wirrinya	8	...	4	2
United States						
US1	...	Illinois	...	...	1	4
US4	...	Illinois	...	...	4	5
US5	...	Illinois	...	...	5	6
US7	...	Illinois	...	...	7	7
US8	...	Illinois	...	...	8	8
US9	...	Illinois	...	...	9	9
US10	...	Illinois	...	...	10	4
US13	...	Illinois	...	...	13	4
US15	...	Illinois	...	...	15	10
US21	...	Illinois	...	...	21	11
US22	...	Illinois	...	...	22	12
US25	...	Illinois	...	...	25	13
904(4)WIS	...	Wisconsin	...	...	4	14
W18750N	...	Wisconsin	...	...	4	15
W18762N	...	Wisconsin	...	...	...	10

a 6-base recognition sequence): *Bam*HI, *Pst*I, *Hin*dIII, *Eco*RI, *Kpn*I, *Eco*RV, *Pvu*II, and *Xho*I against a subset of five Australian and five U.S. isolates of *P. sojae*. For this study, two of these cDNA clones, *Pmg*C1 and *Pmg*C12, and three random genomic clones, p*Pmg*S2, p*Pmg*S15, and p*Pmg*S19, were selected that showed clearly identifiable polymorphisms using the restriction enzymes *Kpn*I and *Xho*I. DNA probes were ^{32}P-labeled as described by Whisson et al. (30), and hybridizations were carried out in a Techne hybridizer (Techne Ltd., Cambridge, England) oven at 65°C using 0.5 M Na_2HPO_4/NaH_2PO_4, pH 7.2, 1 mM EDTA, and 7% sodium dodecyl sulfate as hybridization buffer. Filter washing, autoradiography, and stripping of blots for reuse was as described by Whisson et al. (30).

Data analysis. A multilocus RFLP genotype was derived for each isolate based on all fragments observed with five selected RFLP probes. The multilocus RFLP genotypes were based on the presence or absence of RFLP fragments, and each fragment was assumed to represent a single genetic locus. Isolates with the same multilocus RFLP genotype were considered clonal. In the case of *Pmg*C1 and *Pmg*C12, only two fragments were observed that were confirmed to be allelic after genetic analysis (S. C. Whisson, *unpublished data*). A measure of genotypic diversity (24) was calculated on the basis of the number of multilocus RFLP genotypes in each region. Genotypic diversity in each region was measured using the formula

$$\hat{G} = \frac{1}{\sum_{x=0}^{N}[(f_x)\cdot(x/N)^2]}$$

where N is the sample size and f_x is the number of distinct multilocus RFLP genotypes observed x times in each region. The maximum possible value for $\hat{G}$, which occurs when each individual in a region has a different genotype, is the number of individuals in each region. To compare $\hat{G}$ between regions with different sample sizes, we divided $\hat{G}$ from each region by N according to McDonald et al. (15) to calculate the percentage of maximum possible diversity that was obtained.

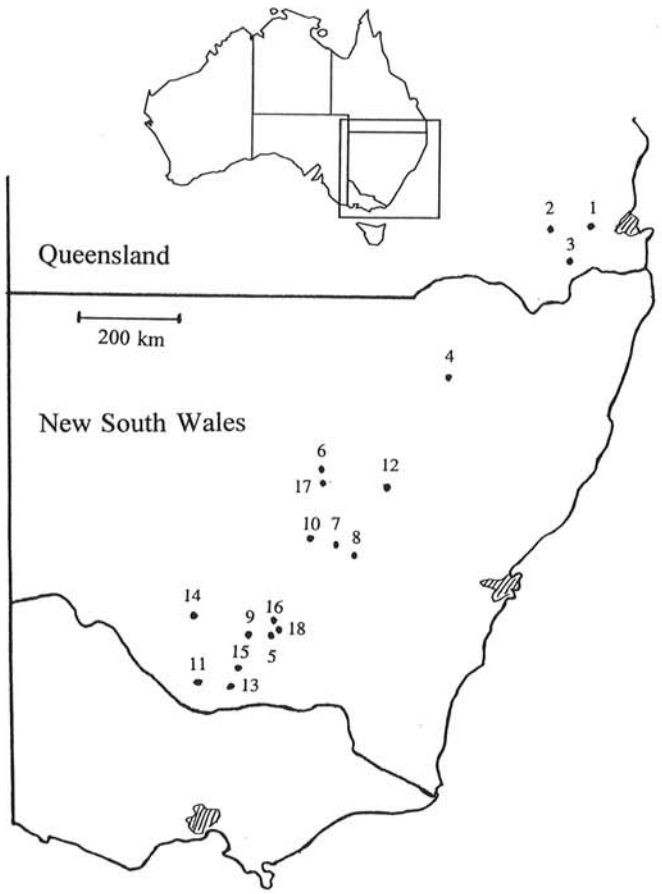

Fig. 1. Map of southeastern Australia, with inset of Australia, showing the location of *Phytophthora sojae* isolate collection sites in numbers corresponding to the location numbers in Table 1.

RESULTS

In the Australian population, virulence was observed against soybean resistance genes *Rps*1a, 1c, 3a, 5, 6, and 7 (Table 2) distributed among five races (1, 4, 13, 15, and X). Race 1 was collected from all three regions (Table 1), and races 4 and 15 were found only in southeastern Queensland and southern New South Wales. Race 13 was detected only once, in southeastern Queensland, and a previously undescribed race (X) also was identified once in southern New South Wales. Race X shows virulence toward *Rps*1a, 1c, 3a, 5, and 7 (Table 2) and has not yet been identified elsewhere in the world.

Hybridization of Southern blots containing *P. sojae* DNA digested with restriction endonucleases *Xho*I and *Kpn*I with the five RFLP probes detected 30 fragments of nuclear genomic DNA among the 84 Australian *P. sojae* isolates (Table 3). Two of the five RFLP probes revealed a total of seven polymorphic fragments (Table 3), which led to the distinction of three multilocus RFLP genotypes (#1, #2, and #3). Hybridization of *Kpn*I blots with probe p*Pmg*S2 showed that 80 Australian isolates possessed four nonpolymorphic fragments, while 4 isolates (CIFR10, UQ296, UQ337, and UQ427 = RFLP genotypes #2 and #3) possessed six additional hybridizing fragments (Fig. 2). The difference between RFLP genotypes #2 and #3 is the presence of one additional fragment in RFLP genotype #3 (CIFR10) after hybridization with probe p*Pmg*S19. RFLP genotype #1 occurred in all three regions investigated in Australia (Table 4). RFLP genotype #2 was identified in three locations in 1991, 1992, and 1993 in southern New South Wales (Fig. 1, locations 17, 10, and 8, respectively). The distinct RFLP genotype #3 was identified once only in 1983, near Bedgerebong in southern New South Wales (Table 1).

Among the 15 isolates from the United States, the five RFLP probes detected 13 polymorphic fragments from a total of 31 observed fragments (Table 3). The 13 polymorphic fragments led to the identification of 12 RFLP genotypes, all unique to U.S. isolates (Table 4). With the exception of the six additional hybridizing fragments observed in RFLP genotypes #2 and #3, after

TABLE 2. Races observed in the Australian *Phytophthora sojae* population, the resistance genes (*Rps*) they are able to overcome, and the restriction fragment length polymorphism (RFLP) genotype(s) identified in each race

Race	*Rps* gene virulent toward	RFLP genotype	No. of isolates
1	7	1, 2, 3	31
4	1a, 1c, 7	1, 2	33
13	6, 7	1	1
15	3a, 5, 7	1	15
X	1a, 1c, 3a, 5, 7	1	1

TABLE 3. The total number of fragments (Frag.) and polymorphic fragments (Poly. frag.) per restriction fragment length polymorphism (RFLP) probe observed in *Phytophthora sojae* populations from Australia and the United States

RFLP probe	Enzyme	Australia		The United States	
		Frag.	Poly. frag	Frag.	Poly. frag.
*Pmg*C1	*Xho*I	1	0	2	2
*Pmg*C12	*Xho*I	1	0	2	2
p*Pmg*S2	*Kpn*I	10	6	5	2
p*Pmg*S15	*Xho*I	10	0	11	3
p*Pmg*S19	*Xho*I	8	1	11	4
Total		30	7	31	13

hybridization with probe p*Pmg*S2 all 24 fragments identified with the five RFLP probes among the Australian isolates also were identified among the limited number of isolates from the United States.

Genotypic diversity was low in the Australian *P. sojae* population according to Stoddart and Taylor's (24) measure of genotypic diversity based on multilocus RFLP genotype data. Only between 2.5 and 14.5% of maximum possible diversity was observed in the three soybean-growing regions in eastern Australia compared to 60% in the United States (Table 5).

All races of *P. sojae* (1, 4, 13, 15, and X) identified so far in Australia occurred in background RFLP genotype #1. Isolates with background RFLP genotype #2 included isolates of races 1 and 4, and RFLP genotype #3 was of race 1 (Table 6). Races collected from the United States included isolates representing many genetic backgrounds (Table 1), e.g., RFLP genotype #4 included isolates of races 1, 10, and 13. Identical races collected in Australia and the United States occurred in different background RFLP genotypes, e.g., race 4 (Australia) in RFLP genotypes #1 and #2 and race 4 (United States) in RFLP genotypes #5, #14 and #15 (Table 1).

DISCUSSION

The genetic variation among isolates of *P. sojae* from two geographically isolated populations (Australia and the United States) was explored in this investigation. Polymorphic RFLP probes detected a low level of genotypic diversity among a large set of isolates collected between 1979 and 1993 in eastern Australia. More than 95% of the Australian *P. sojae* population had the same multilocus RFLP background genotype and displayed a subset of the DNA fragments also observed in the U.S. population. These observations indicate a low level of initial genetic variation in the founding population of *P. sojae* in Australia. It is likely that only one or at most a few genetically closely related race 1 genotypes of *P. sojae* were introduced to Australia. This introduced race spread quickly to all soybean-growing areas of eastern Australia. A high percentage (95%) of clonal isolates (isolates with the same multilocus RFLP genotype) in a population collected over a number of years in a geographically large area such as eastern Australia indicates that the *P. sojae* population has remained nearly monomorphic since its introduction to Australia.

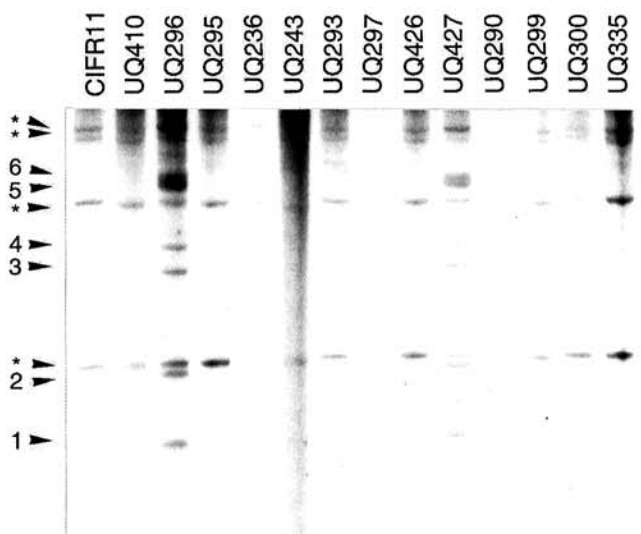

Fig. 2. Autoradiogram showing DNA fragments of 14 isolates of *Phytophthora sojae* detected by hybridization of probe p*Pmg*S2 to DNA digested with *Kpn*I. Asterisks indicate monomorphic DNA fragments in the Australian *P. sojae* population. The additional polymorphic fragments, identified in the isolates of restriction fragment length polymorphism genotypes #2 and #3 are numbered 1 to 6.

Stoddart and Taylor's (24) measure of genotypic diversity, based on multilocus RFLP genotypes, ranged between 2.5 and 14.3% of maximum possible diversity in the three regions in eastern Australia. The difference in genotypic diversity between populations from different regions in Australia is probably due to the small sample size obtained from northern New South Wales (Table 5) in which only one RFLP genotype was identified (Table 4). The high level of genotypic diversity observed in the United States compared to Australia can be explained in part by the preselection of different races for this study as well as higher levels of genetic variation present in the *P. sojae* population from the United States, as was observed by Förster et al. (5).

Probe p*Pmg*S2 was of particular interest because it detected a highly specific and complex RFLP genotype for isolate CIFR10 (RFLP genotype #3) collected in 1983 (30). This hybridization pattern for probe p*Pmg*S2 was identified again in 1991 (UQ296), 1992 (UQ337), and 1993 (UQ427) (Fig. 2) in RFLP genotype #2 in southern New South Wales (Fig. 1, locations 17, 10, and 8, respectively). The only difference between RFLP genotypes #2 and #3 was one additional fragment after hybridization with probe p*Pmg*S19 for RFLP genotype #3. Therefore, it appears that RFLP genotype #2 arose from RFLP genotype #3 through mutation and that isolates with the unique hybridization pattern observed for probe p*Pmg*S2 have spread locally since it was first identified in Bedgerebong (Fig. 1, location 7) in southern New South Wales in 1983. Since no additional polymorphisms were observed between the Australian *P. sojae* isolates, it is possible that RFLP genotypes #2 and #3 arose from RFLP genotype #1 and subsequently mutated for virulence. Alternatively, a new introduction from overseas could have taken place.

The race structure of the Australian *P. sojae* population, with three common races (1, 4, and 15) and two single-isolate races (13 and X), can be attributed largely to the commercial cultivation of resistant cultivars. Currently, most of the variation for virulence was observed in southeastern Queensland and southern New South Wales, and only race 1 has been isolated from a limited number of sites in northern New South Wales. In New South Wales,

TABLE 4. Regional distribution of *Phytophthora sojae* restriction fragment length polymorphism (RFLP) genotypes and races observed in Australia and the United States

Region	No. of isolates	RFLP genotype	Race
Southeastern Queensland	30	1	1, 4, 13, 15
Northern New South Wales	7	1	1
Southern New South Wales	47	1, 2, 3	1, 4, 15, X
The United States	15	4-15	1, 4, 5, 7, 8, 9, 10, 13, 15, 21, 22, 25

TABLE 5. Genotypic diversity and the percentage of maximum possible diversity obtained in populations of *Phytophthora sojae* from different regions in Australia and the United States

Region	No. of isolates	No. of RFLP genotypes	$\hat{G}$	$(\hat{G}/N)$ %
Southeastern Queensland	30	1	1	3.3
Northern New South Wales	7	1	1	14.3
Southern New South Wales	47	3	1.2	2.5
The United States	15	12	9	60.0

TABLE 6. The number of isolates per restriction fragment length polymorphism (RFLP) genotype and the different races observed per RFLP genotype among 84 isolates of *Phytophthora sojae* in Australia

RFLP genotype	No. of isolates	Race
1	80	1, 4, 13, 15, X
2	3	1, 4
3	1	1

the appearance and rapid spread of race 4 has been attributed to soybean production based on cultivars Ridley and Lachlan (21). From Table 1 it is apparent that four races (1, 4, 13, and 15) were identified at Hermitage Research Station, Warwick, Condobolin (1 and 4), and Finley (1, 15, and X), where soybean evaluation trials are conducted. In these three locations (Fig. 1, locations 3, 10, and 13, respectively), soybean cultivars with new *Rps* genes are grown in short rotations and are evaluated for their resistance to Phytophthora root rot, leading to the selection of new races. In southeastern Queensland, only races 1 and 15 have been found in commercial crops where soybean production based on cultivar Davis has led to a rise in the importance of race 15 (20). In southeastern Queensland, races 4 and 13 have been confined to experimental field sites, and these locations could be used experimentally to evaluate the influence of deployment of different *Rps* genes in soybean on the evolution of new races, providing insights into the capacity to predict the appearance of particular races.

There are several possible mechanisms to explain the occurrence of new races of *P. sojae* in Australia since the identification of the first isolate (race 1) in 1979 (17), including the presence of an indigenous wild-type population, the sequential introduction of races from overseas to Australia, mutation in a common genetic background, and genetic recombination (sexual and parasexual). The presence of an indigenous wild-type population of *P. sojae* in Australia seems unlikely since a higher level of genotypic diversity than currently is observed would be expected and would be more divergent from the population present in the United States (4,5,30). Moreover, *P. sojae* was detected for the first time in Australia in 1979, a decade after soybeans were first grown on a relatively large-scale commercial basis.

Sequential introduction of new races into Australia seems unlikely based on the low level of genotypic diversity in the Australian population and the fact that over 95% of the isolates collected in Australia belong to RFLP genotype #1. From our data, it is apparent that *P. sojae* was introduced and that mutations in avirulence genes after the introduction led to the occurrence of new races of *P. sojae* in Australia. Our results and those of others demonstrate a high level of genotypic diversity in the U.S. population for this pathogen which led Förster et al. (5) to hypothesize that *P. sojae* is indigenous to the United States. However, a population genetic study on a global scale is required to test this hypothesis.

RFLP genotype #1 includes isolates of all races identified in Australia, which provides evidence for the hypothesis that these races evolved locally, through mutation, from the original introduced genotype. Genetic analysis of virulence/avirulence in *P. sojae* recently performed in our laboratory revealed that avirulence is dominant over virulence in *P. sojae* for the seven avirulence genes analyzed (27,28). In the ascomycete *Cladosporium fulvum* a 1-bp mutation was sufficient to change the phenotype from avirulent to virulent, and convergent evolution was demonstrated when different isolates, both virulent toward the *Cf*4 resistance gene in tomato, showed different mutations in the *Avr*4 gene (10). In addition to rapid spread of fungal spores, convergent evolution might explain the rapid development of new races of many plant pathogens in different parts of the world when new resistance genes are deployed.

The occurrence of a low percentage (<5%) of outcrossing in vitro for *P. sojae* (2,5,27) suggests the possibility that new races may arise by outcrossing among existing races in field situations. Thus, with avirulence dominant, outcrossing between different races and subsequent selfing of the hybrids may produce new races. Population genetic data collected by Förster et al. (5) provides evidence for low levels of outcrossing in field situations in the United States. However, in vivo experiments are needed to confirm that outcrossing is possible under field conditions. The possible occurrence of outcrossing among different races of the homothallic pathogen *P. sojae* in the field may counter breeding efforts aimed at controlling Phytophthora root rot in soybean. Selfing and low levels of outcrossing among identical or closely related genotypes will not give rise to large numbers of polymorphic markers in such a population (12). Selfing, asexual reproduction, and outcrossing are distinct modes of reproduction that can be easily distinguished using molecular markers (3,12). However, in the case of *P. sojae* in Australia, the founding population appears to have a very narrow genetic base with a paucity of polymorphic markers, which may make it difficult to distinguish between these modes of reproduction in this homothallic plant pathogen.

LITERATURE CITED

1. Athow, K. L. 1986. Phytophthora root rot of soybean. Pages 418-426 in: Proc. 3rd World Soybean Res. Conf. R. Shibles, ed. Westview Press, Boulder, CO.
2. Bhat, R. G., and Schmitthenner, A. F. 1993. Genetic crosses between physiologic races of *Phytophthora sojae*. Exp. Mycol. 17:122-129.
3. Drenth, A., Tas, I. C. Q., and Govers, F. 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. Eur. J. Plant Pathol. 100:97-107.
4. Förster, H., Kinscherf, T. G., Leong, S. A., and Maxwell, D. P. 1988. Restriction fragment length polymorphisms of the mitochondrial DNA of *Phytophthora megasperma* isolated from soybean, alfalfa, and fruit trees. Can. J. Bot. 67:529-537.
5. Förster, H., Tyler, B. M., and Coffey, M. D. 1994. *Phytophthora sojae* races have arisen by clonal evolution and by rare outcrosses. Mol. Plant-Microbe Interact. 7:780-791.
6. Francis, D. M., and St. Clair, D. A. 1993. Outcrossing in the homothallic oomycete, *Pythium ultimum*, detected with molecular markers. Curr. Genet. 24:100-106.
7. Hansen, E. M., and Maxwell, D. P. 1991. Species of the *Phytophthora megasperma* complex. Mycologia 83:376-381.
8. Heritage, A. D., Castles, C. J., and Pierpoint, J. A. 1993. Surveys of Phytophthora root rot and stem rot in early maturing soybean crops in southern temperate regions of Australia from 1985 to 1988. Austr. Plant Pathol. 22:131-136.
9. Irwin, J. A. G., and Dale, J. L. 1982. Relationships between *Phytophthora megasperma* isolates from chickpea, lucerne, and soybean. Austr. J. Bot. 30:199-210.
10. Joosten, M. H. A. J., Cozijnsen, T. J., and De Wit, P. J. G. M. 1994. Host resistance to a fungal tomato pathogen lost by a single base-pair change in the avirulence gene. Nature 367:384-386.
11. Keeling, B. L. 1984. A new physiologic race of *Phytophthora megasperma* f. sp. *glycinea*. Plant Dis. 68:626-627.
12. Kohn, L. M. 1995. The clonal dynamic in wild and agricultural plant pathogen populations. Can. J. Bot. (Suppl. 1) 73:1231-1240.
13. Layton, A. C., Athow, K. L., and Laviolette, F. A. 1986. New physiologic race of *Phytophthora megasperma* f. sp. *glycinea*. Plant Dis. 70: 500-501.
14. Liew, E. C. Y., Maclean, D. J., Manners, J. M., Dawson, D., and Irwin, J. A. G. 1991. Use of restriction fragment length polymorphisms to study genetic relationships between Australian and Japanese isolates of *Phytophthora vignae*. Austr. J. Bot. 39:335-346.
15. McDonald, B. A., Miles, J., Nelson, L. R., and Pettway, R. E. 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. Phytopathology 84:250-255.
16. Nygaard, S. L., Elliot, C. K., Cannon, S. J., and Maxwell, D. P. 1989. Isozyme variability among isolates of *Phytophthora megasperma*. Phytopathology 79:773-780.
17. Pegg, K. G., Kochman, J. K., and Vock, N. T. 1980. Root and stem rot of soybean caused by *Phytophthora megasperma* f. sp. *glycinea* in Queensland. Austr. Plant Pathol. 9:15.
18. Ribiero, O. K. 1978. A Source Book of the Genus *Phytophthora*. J. Cramer, Vaduz, Leichtenstein.
19. Rose, J. L., Irwin, J. A. G., Ryley, M. J., Langdon, P. W., and Jenner L. B. 1982. Reaction of soybean cultivars to races of *Phytophthora megasperma* f. sp. *glycinea* in Queensland. Austr. J. Agric. Res. 33:763-771.
20. Ryley, M. J., and Obst, N. R. 1991. Race-specific resistance in soybean cv. Davis to *Phytophthora megasperma* f. sp. *glycinea*. Plant Dis. 76:665-668.
21. Ryley, M. J., Obst, N. R., and Stovold, G. E. 1991. A new race of *Phytophthora megasperma* f. sp. *glycinea* on soybean in Australia. Austr. Plant Pathol. 20:97-100.
22. Schmitthenner, A. F. 1985. Problems and progress in control of Phytophthora root rot of soybean. Plant Dis. 69:362-368.
23. Schmitthenner, A. F. 1989. Phytophthora rot. Pages 35-38 in: Compendium of Soybean Diseases. 3rd ed. P. A. Backman and J. B. Sinclair, eds. The American Phytopathological Society, St. Paul, MN.

24. Stoddart, J. A., and Taylor, J. F. 1988. Genotypic diversity: Estimation and prediction in samples. Genetics 118:705-711.
25. Troedson, R. J., Ryley, M. J., Byth, D. E., and Irwin J. A. G. 1991. Effect of Phytophthora root and stem rot on the response of field-grown soybean to saturated soil culture. Austr. J. Agric. Res. 42:791-800.
26. Ward, E. W. B. 1990. The interaction of soybean with *Phytophthora megasperma* f. sp. *glycinea*: Pathogenicity. Pages 311-327 in: Biological Control of Soilborne Plant Pathogens. B. Hornby, ed. CAB International, Wallingford, England.
27. Whisson, S. C., Drenth, A., Maclean, D. J., and Irwin, J. A. G. 1994. Evidence for outcrossing in *Phytophthora sojae* and linkage of a DNA marker to two avirulence genes. Curr. Genet. 27:77-82.
28. 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.
29. Whisson, S. C., Liew, E. C. Y., Howlett, B. J., Maclean, D. J., Manners, J. M., and Irwin, J. A. G. 1993. An assessment of genetic relationships between members of the *Phytophthora megasperma* complex and *Phytophthora vignae* using molecular markers. Austr. J. Syst. Bot. 6:295-308.
30. Whisson, S. C., Maclean, D. J., Manners, J. M., and Irwin, J. A. G. 1992. Genetic relationships among Australian and North American isolates of *Phytophthora megasperma* f. sp. *glycinea* assessed by multicopy DNA probes. Phytopathology 82:863-868.
31. Yoon, C. S., Glawe, D. A., and Shaw, P. D. 1991. A method for rapid small-scale preparation of fungal DNA. Mycologia 83:835-838.