High frequency mitotic gene conversion in genetic hybrids of the oomycete *Phytophthora sojae*

Jureerat Chamnanpunt, Wei-xing Shan*, and Brett M. Tyler†

Department of Plant Pathology, University of California, Davis, CA 95616

Edited by David D. Perkins, Stanford University, Stanford, CA, and approved October 5, 2001 (received for review August 30, 2001)

Microbial populations depend on genetic variation to respond to novel environmental challenges. Plant pathogens are notorious for their ability to overcome pesticides and host resistance genes as a result of genetic changes. We report here that in particular hybrid strains of *Phytophthora sojae*, an oomycete pathogen of soybean, high frequency mitotic gene conversion rapidly converts heterozygous loci to homozygosity, resulting in heterokaryons containing highly diverse populations of diploid nuclei. In hybrids involving strain P7076, conversion rates of up to 3×10^{-2} per locus per nucleus per generation were observed. In other hybrids, rates were of the order of 5×10^{-5}. Independent gene conversion was observed within a selected linkage group including loci as close as 0.7 kb apart and in unlinked markers throughout the genome. Gene conversions continued throughout vegetative growth and were stimulated by further sexual reproduction. At many loci, conversion showed extreme disparity, with one allele always being lost, suggesting that conversion was initiated by allele-specific double-stranded breaks. Pedigree analysis indicated that individual loci undergo multiple independent conversions within the nuclei of a vegetative clone and that conversion may be preceded by a heritable "activation" state.

The ability of microbial populations to respond to novel challenges from their environment depends on their mechanisms for generating genetic variation. Plant pathogen populations are notorious for their ability to overcome pesticides and host resistance as a result of the rapid appearance of new genetic types (1–3). In addition to universal mechanisms such as genetic exchange and transposon-induced variation, microbes display a variety of novel mechanisms for generating genetic variation such as high frequency deletions (4), optional chromosomes (5, 6), phase variation due to recombination (7), and telomere switching (8).

Plants evolve defense receptors that detect molecules produced by pathogens. The plant genes encoding these receptors are termed "resistance genes," and the pathogen genes encoding the detected molecules are termed "avirulence" genes (9, 10). Avirulence is dominant in the pathogen, because the presence of the incriminating pathogen molecule triggers a rapid and effective defense response when a plant variety that carries the appropriate resistance gene is infected. The presence of resistance genes in a plant population thus creates selection pressure in favor of novel "races" of the pathogen that no longer express the relevant avirulence gene. Losses of avirulence genes in fungal pathogens have occurred by partial or complete deletions (10–13) or point mutations (11, 14). Bacterial avirulence genes have been lost by point mutations (9, 15), deletions (9), transposon insertions (16), or loss of the vector plasmid (9). In oomycetes, the loss of transcription of avirulence genes has been observed in two cases, elicitins (17) and *Phytophthora sojae Avr1b* (W.-x.S. and B.M.T., unpublished data).

Oomycetes resemble true fungi morphologically and physiologically (2) but are related most closely to golden brown algae such as diatoms (18). They grow as coenocytic hyphae and produce several kinds of asexual spores including mononucleate zoospores. Many oomycetes including *Phytophthora* species cause serious diseases on crops (2). *P. sojae* infects soybean. More than 13 resistance genes have been identified in soybean against *P. sojae* (19), and avirulence genes corresponding to most of them have been identified in *P. sojae* (20, 21). Several *Phytophthora* species show a remarkable degree of variation even during asexual reproduction. During serial subculture of *Phytophthora infestans* on detached potato leaves, avirulence in the presence of host resistance gene *R4* was lost without selection as early as the third or fourth passage (22). In the field, the rapid appearance of new races of *P. infestans* has made single major resistance genes useless in potato (23). Resistance against the fungicide metalaxyl has arisen rapidly in *P. infestans* and other oomycetes (24–26). In *P. sojae*, changes from avirulence to virulence and *vice versa* were observed among two generations of single zoospore lines (27).

Phytophthora species are typically diploid and can reproduce sexually (2). Meiosis occurs in male and female gametangia. A diploid sexual oospore is formed when a female gametangium is fertilized by a haploid nucleus from a male gametangium. *P. sojae* is homothallic and freely produces oospores in pure culture (28). Outcrossing can occur when two strains are mixed in culture (20, 21, 29, 30) or infect the same plant (31). There are four major genotypes of *P. sojae*, and rare outcrosses among these genotypes in the field have resulted in much of the variation in the species (28). In the laboratory, outcrosses have been used to create genetic maps consisting of molecular markers and to identify and map avirulence genes (20, 21).

While creating a genetic map spanning the avirulence gene *Avr1b* of *P. sojae* (W.-x.S. and B.M.T., unpublished data), we noticed that particular molecular markers changed occasionally from heterozygosity to homozygosity during vegetative growth. The same mechanism, occurring at high frequency, potentially explained extreme biases in the transmission of many unlinked markers that we had observed previously in a cross between strains P6497 and P7076 (21). To test our hypothesis directly and determine the mechanism of the change to homozygosity, we examined molecular genetic markers within and outside a selected linkage group in a large population of somatic segregants.

Materials and Methods

Strains and Genetic Methods. Strains P6497, P7076, and P7064 represent genotypes I, II, and IV, respectively (28). For outcrosses, single germinating oospores from mixed cultures of parent strains were selected by microscopy and cultured in 5-cm culture dish wells containing V8 agar (21). F_1 hybrids were identified by using random amplified polymorphic DNA (RAPD; ref. 32) markers (21). For selfing, oospores were selected from pure cultures. For the vegetative passaging experiment, a frozen agar plug of each strain was recovered from liquid nitrogen storage, inoculated onto a 9-cm Petri dish containing cleared V8 agar, and incubated for 7 days at 25°C. Four agar plugs then were cut from each plate, inoculated onto four separate 9-cm plates, and incubated for 7 days, resulting

This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: RAPD, random amplified polymorphic DNA.

*Present address: Research School of Biological Sciences, Australian National University, PO Box 475, Canberra Act 2601, Australia.

†To whom reprint requests should be addressed at: Department of Plant Pathology, One Shields Avenue, University of California, Davis CA 95616. E-mail: bmtyler@ucdavis.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

www.pnas.org/cgi/doi/10.1073/pnas.251464498

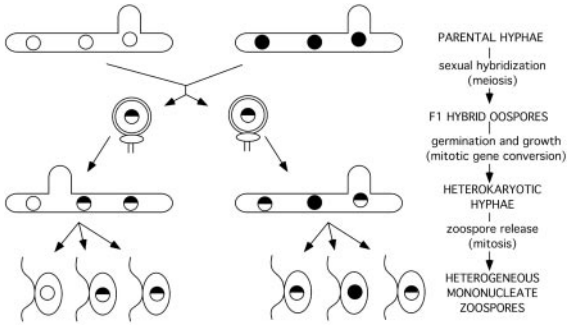

Fig. 1. Analysis of mitotic changes by isolation of zoospores. The design of the experiment is shown together with anticipated changes in genetic markers. Open and filled circles denote nuclei homozygous for a given polymorphic marker. Half-filled circles denote heterozygous nuclei. Oospores are diploid sexual spores and result from the fusion of haploid gametangia. Zoospores are mononucleate vegetative spores, the nuclei of which are generated by mitosis.

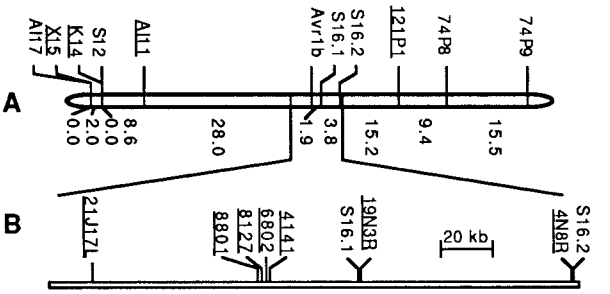

Fig. 2. Genetic and physical maps of the linkage group analyzed. (*A*) Genetic map. The map was constructed by analysis of 100 F_2 progeny from a cross of P6497 × P7064 using MAPMAKER 1.0. All molecular markers are RAPD markers except 121P1, 74P8, and 74P9, which are restriction fragment length polymorphisms. Codominant alleles of the underlined markers were used for this study. *Avr1b* is a phenotypic marker. Distances are in centimorgans. (*B*) Physical locations of markers on a 200-kb bacterial artificial chromosome contig spanning *Avr1b*. Underlined markers were used in this study. Marker 8127 lies within *Avr1b*. The expansion lines show the position and orientation of the contig on the genetic map.

in four independent subcultures. A single plug from each subculture then was inoculated onto a fresh 9-cm plate and incubated for 7 more days. This process was repeated three more times. To initiate zoospore production, a 9-cm Petri dish containing cleared V8 agar was inoculated with a single agar plug from the original 5-cm oospore culture in the case of the F_1 and F_2 hybrids (F_1 hybrids NC15A and NC15B originated from different agar plugs at this point) or from the final passage plate in the case of the subculturing experiment. After 7 days, zoospore release was stimulated by repeated washing of the culture (33). After harvest, single germinating zoospores were selected by microscopy and cultured in 5-cm wells. To isolate DNA for analysis, a single plug from the original culture of the zoospore line was used to inoculate 5 ml of liquid V8 medium. After 5 days of growth, a small piece of mycelium was removed and DNA extracted (33) from it for scoring of molecular markers. Five days' growth corresponds approximately to 20 rounds of nuclear division.

Genetic Markers. Marker 121P1 was identified previously as a restriction fragment length polymorphism (21, 28). Markers X15, K14, and AI11 were identified initially as RAPD markers (W.-x.S. and B.M.T., unpublished data). The region corresponding to the polymorphic fragments in each case was cloned from strains P6497, P7076, and P7064 and sequenced. Markers 21J17L, 19N3R, and 4N8R were derived from the ends of bacterial artificial chromosome clones (W.-x.S. and B.M.T., unpublished data). In each case the relevant fragment was cloned and sequenced from all three strains. Markers 4141, 6802, and 8127 were identified from sequencing the right end of bacterial artificial chromosome 1C13. 8127 lies within the *Avr1b* gene (W.-x.S. and B.M.T., unpublished data). To create codominant genetic markers scorable by the PCR, primers were designed from the relevant sequences that could amplify a fragment from all three strains. Polymorphisms were scored by digestion with an appropriate restriction enzyme except for markers 121P1, AI11, and X15, for which single strand conformation polymorphism (34) was used to detect polymorphisms between strains P6497 and P7076. The markers P2, M4, AP4, A19, and B16 are all uncloned RAPD markers and were scored by using the corresponding Operon Technologies (Alameda, CA) primers (www.operon.com) using the amplification conditions of Whisson *et al.* (20). The sequences of all primers, amplification conditions, and choices of restriction enzyme are available in Table 5 and additional text, which are published as supporting information on the PNAS web site, www.pnas.org.

Results

High Frequency Changes to Homozygosity in F_1 Progeny of P6497 × P7076. To test for changes to homozygosity, we examined molecular genetic markers from a linkage group spanning aviru-

Table 1. Conversion of heterozygous markers to homozygosity in F_1 hybrids from P6497 × P7076

F_1 hybrid	Lines analyzed	Number of zoospore lines homozygous for each marker*													
		X15	K14	AI11	21J17L	8801	8127	6802	4141	19N3R	4N8R	121P1	AP4	AP19	B16
NC 162	36	0	0	0	0	0	0	0	0	10 (7)	0	0	0	5 (7)	0
NC 174	22	0	0	0	0	0	0	0	0	16 (7)	0	0	1 (6)	3 (7)	0
NC 123	33	0	1 (6)	0	0	15 (6)	13 (6)	0	6 (6)	25 (7)	25 (6)	0	4 (6)	8 (7)	4 (6)
NC 64	26	0	3 (6)	1 (7)	0	0	11 (6)	0	7 (6)	15 (7)	21 (6)	0	0	6 (7)	6 (6)
NC 111	21	0	0	0	0	0	1 (6)	0	0	13 (7)	0	0	0	1 (7)	1 (6)
NC 160	28	0	0	1 (7)	0	9 (7)	0	0	0	8 (7)	0	0	0	0	1 (7)
NC 15A	29	2 (7)	0	1 (7)	0	24 (6)	0	0	6 (6)	4 (7)	0	0	0	0	4 (7)
NC 15B	20	1 (6)	0	2 (7)	8 (6)	9 (6)	0	0	5 (6)	17 (7)	0	0	0	0	6 (7)
NC 16	19	0	0	1 (6)	0	4 (7)	0	0	0	16 (7)	0	0	0	0	0
NC 67	22	0	0	1 (6)	0	4 (7)	0	0	0	2 (7)	0	0	0	0	0
Total	256	1 (6)	4 (6)	2 (6)	8 (6)	48 (6)	25 (6)	0 (6)	24 (6)	0 (6)	46 (6)	0 (6)	5 (6)	0 (6)	11 (6)
		2 (7)	0 (7)	5 (7)	0 (7)	17 (7)	0 (7)	0 (7)	0 (7)	126 (7)	0 (7)	0 (7)	0 (7)	23 (7)	11 (7)

All markers are codominant except AP19 and AP4, which are dominant. AP4, AP19, and B16 are unlinked to all other markers or to each other. All others are linked (Fig. 2). Unlinked dominant RAPD markers P2 and M4 showed no changes among any zoospore lines. NC15A and NC15B are different subcultures of the same F_1 individual.

*The number of lines homozygous for the P7076 allele (7) and the P6497 allele (6).

Table 2. Genotypes of zoospores lines from F_1 hybrid NC123

		Genotypes of zoospore lines†								
Row	Lines*	8801	8127	6802	4141	19N3R	4N8R	AP4	AP19	B16
1	3	h	h	h	h	7	6	h	H‡	h
2	3	h	h	h	h	7	h	h	H	h
3	2	6	h	h	h	7	6	h	H	h
4	2	6	6	h	6	7	6	h	H	h
5	2	6	h	h	h	7	6	6	H	h
6	2	h	h	h	6	h	h	h	H	h
7	2	6	6	h	h	7	6	h	H	h
8	2	h	h	h	h	h	6	h	H	h
9	1	h	6	h	6	7	6	h	H	h
10	1	h	h	h	h	7	6	6	H	h
11	1	h	6	h	h	h	6	h	6	h
12	1	6	h	h	6	7	6	h	H	h
13	1	6	6	h	h	h	6	h	6	h
14	1	6	6	h	h	7	6	h	6	6
15	1	h	6	h	h	7	h	h	6	6
16	1	h	6	h	h	h	6	h	H	h
17	1	6	6	h	h	7	6	h	H	6
18	1	h	h	h	h	7	6	h	6	h
19	1	6	h	h	h	7	6	6	H	6
20	1	h	6	h	h	7	6	h	6	h
21	1	h	6	h	h	7	h	h	H	h
22	1	h	h	h	h	7	h	h	6	h
23	1	6	h	h	h	h	6	h	6	h

The markers are as described in Table 1. Markers changed in one or no lines are not shown. A total of 33 lines were analyzed.

*The number of zoospore lines having the given genotype.

†h, heterozygous; 6, homozygous for P6497 allele; 7, homozygous for P7076 allele.

‡Because AP19 is dominant, H indicates heterozygous or homozygous for P7076 allele.

lence gene *Avr1b* (W.-x.S. and B.M.T., unpublished data) as well as randomly chosen markers unlinked to *Avr1b*. Somatic segregants were produced by isolating 20–40 mononucleate zoospores each from freshly prepared F_1 hybrids from two crosses: P6497 × P7064 and P6497 × P7076 (Fig. 1). The first cross displays normal Mendelian genetics (21), and we previously observed few somatic changes in progeny from this cross. The second cross displays extreme biases in marker segregation (21), and thus we predicted a high frequency of somatic changes in progeny from this cross. We isolated the zoospore lines as early as possible after oospore germination and isolated DNA as early as possible after germination of the zoospores to minimize the number of changes that might accumulate. We examined the fate of 11 codominant genetic markers linked to *Avr1b* (Fig. 2) and five unlinked markers. Seven of the linked markers derived from a 200-kb bacterial artificial chromosome contig spanning the *Avr1b* gene (W.-x.S. and B.M.T.). All the markers were homozygous in the three strains involved.

Among a total of 351 zoospore lines from 15 F_1 progeny from P6497 × P7064, only two showed a change to homozygosity for any marker: K14 in both cases (data not shown). In contrast, 180 of 256 zoospore lines from P6497 × P7076 showed changes in at least one marker, affecting all nine F_1 individuals (Table 1). Four markers showed no changes. Among the markers that changed, the frequency varied from 1 (X15) to 50% (19N3R) of the zoospore lines, which equates to rates of 5×10^{-4}–3×10^{-2} per locus per nucleus per generation, respectively. The changes were not technical artifacts, because they were not observed in segregants from the P6497 × P7064 cross and they could be reproduced by using new DNA preparations. Moreover, for several markers (e.g., 21J17L, 8801, 19N3R, and 4N8R) heterozygosity could be distinguished readily from incomplete restriction enzyme digestion, because the enzyme produced multiple fragments for each allele. There was considerable variation in the frequency of changes among different F_1 individuals. For example, only seven changes were observed among 22 zoospore lines from F_1 hybrid NC67, whereas 95 changes were observed among 36 lines from F_1 hybrid NC123. Some markers changed at high frequency in some F_1 lines but not at all in others (e.g., markers 8127 and 4N8R). Markers both linked and unlinked to *Avr1b* were affected. An unexpected feature of the changes was that the changes at many loci showed extreme disparity‡ as to which allele was lost. For example, marker 19N3R changed in almost 50% of all zoospore lines, but always became homozygous for the allele from P7076. The observed disparity does not result from clonal expansion after a change to homozygosity in a single nucleus, because pedigree analysis shows that most changes have occurred more than once among a set of zoospore lines (see below). Even when changes in favor of either allele were observed, F_1 individuals usually lost one allele but not the other (e.g., at markers 8801, B16, and AI11); an exception was the loss of different alleles of locus X15 between subcultures NC15A and NC15B. Overall, these data demonstrate that high frequency changes to homozygosity occur in progeny of P6497 × P7076 and at relatively low frequency in progeny of P6497 × P7064.

Homozygosity Results from Gene Conversion Without Crossing Over. To infer the mechanism of change, we compared the changes in neighboring markers within individual zoospore lines. Table 2 shows representative data from F_1 progeny NC123, in which changes occur at high frequency. In NC123 the only cases of coconversion of adjacent markers in the linkage group occur between markers 8801 and 8127, which are 674 bp apart. Even adjacent markers 4141, 6802, and 8127, which are 2.7 and 1.3 kb apart, respectively, show no coconversion. Examples of coconversion of adjacent markers are rare in the other progeny also. The lack

‡We use the term *disparity* here to indicate that gene conversion occurs primarily in favor of one allele (54). The terms *polarity* and *directionality* are used sometimes to describe this phenomenon, but these terms are used also to describe bias in the position of a conversion tract relative to the site of its initiation.

Table 3. Frequency of gene conversion after vegetative passaging and sexual reproduction

	F_1 individual		F_1 subculture		F_2 progeny	
Strain*	Lines†	Frequency‡	Lines	Frequency	Lines	Frequency
NC174	1 × 22	0.076 a	4 × 25	0.043 b	4 × 20	0.071 a
NC123	1 × 33	0.15 a	4 × 25	0.12 a	2 × 20	0.28 b
NC15B	1 × 20	0.15 a	4 × 25	0.32 b	6 × 20	0.40 c

*F_1 individual refers to the strains analyzed in Table 1. The frequencies in F_1 subcultures are averages of four independent cultures of the F_1 individual passaged five times. F_2 progeny are 2–6 progeny obtained by selfing the F_1 individual.

†$m \times n$ indicates that n zoospore lines were analyzed from each of m individuals, subcultures, or F_2 progeny.

‡The frequency of conversions per locus per zoospore line averaged among 2–6 individuals including loci with no changes and excluding loci homozygous in the parent F_1 zoospore line (NC174.6, NC123.10, or NC15B.4). Twelve loci were analyzed in NC174 and its derivatives and 10 each for NC123 and NC15B. Comparing across the table, frequencies with the same postscript (a, b, and c) were not significantly different ($P > 0.10$) by the χ^2 test. Frequencies with different postscripts were significantly different ($P < 0.01$).

of coconversion of adjacent markers except for those that are linked most closely argues against any mechanisms for producing homozygosity, such as mitotic crossing over (3, 35, 36) or chromosome loss, that involve large sections of chromosomes. The strong disparity of many of the changes and the fact that alleles in neighboring markers are lost from different parents even in the same zoospore line further supports this conclusion. Therefore we conclude that the mechanism of change is gene conversion without crossing over.

In the cross of P6497 and P7064, which showed only two changes in 351 zoospore lines, the changes nevertheless also seem to have occurred by gene conversion, because the markers closely flanking the site of the two changes, locus K14 (X15 and AI11; Fig. 2), did not change to homozygosity (data not shown). Changes to homozygosity in F_1 hybrids of P6497 and P7064 were observed also in the region surrounding *Avr1a* after extended vegetative culturing (37).

Individual Loci Undergo Multiple Conversions Within Single Clones. Pedigree analysis (28) of the changes observed among the zoospore lines of F_1 individuals reveals that many of the changes have occurred more than once during growth of the F_1. For example, in F_1 hybrid NC123, if the change to homozygosity in marker 8127 had preceded the change in marker 4141, then the change in 4141 could not appear in zoospore lines homozygous for 8127 as well as in lines heterozygous for 8127 unless a change in 4141 occurred at least twice. Table 2 shows that changes in 4141 occur in lines both heterozygous and homozygous for 8127 (compare row 4 with 7 in Table 2). If the change is assumed to have occurred first in 4141, then multiple changes must have occurred in 8127. The same is true relative to 8127 for markers 8801 (compare row 10 with 13 and row 16 with 19), AP19 (row 18 with 21), B16 (row 16 with 19), and indeed for most pairs of markers compared in this way among zoospore lines of NC123 and of the other F_1 progeny.

Gene Conversions Continue During Vegetative Growth. To test whether gene conversions continued during vegetative growth, we selected a single zoospore line from each of three F_1 progeny from P6497 × P7076, namely NC174, which exhibited a low conversion frequency, and NC123 and NC15B, which exhibited a high conversion frequency. Four independent subcultures from each line were passaged five times over a total period of 5 weeks, and then zoospores were isolated and analyzed for homozygosity (Tables 3 and 4). The average frequencies of conversion (conversions per locus per zoospore line) were similar in the NC123.10 subcultures as in the parent F_1 NC123. In NC174.6 the rate was reduced ≈2-fold over NC174, whereas in NC15B.4 the rate was increased ≈2-fold in NC15B. For several loci, conversions did not occur in all four subcultures, suggesting they were new conversion events (e.g., loci 8801, 8127, and B16 in the NC123 derivatives and loci 8127 and 19N3R in the NC15B derivatives). In particular, one single subculture of NC123.10 (NC123.10.D) showed conversions in favor of the P7076 allele of marker B16, whereas four zoospore lines of the NC123 parent showed conversion of B16 in favor of the P6497 allele. The remaining conversions were present in all four subcultures and might have arisen before separation of the subcultures (e.g., at locus 21J17L in the NC123 derivatives and at loci 21J17L, 4141, and 4N8R in the NC15B derivatives).

Gene Conversions Are Stimulated by Sexual Reproduction. To determine whether sexual reproduction stimulated gene conversion

Table 4. Gene conversion during vegetative passage

F_1 hybrid subculture†	Lines analyzed	Lines homozygous for each marker*						
		21J17L	8801	8127	4141	B16	19N3R	4N8R
NC123	33	0	15 (6)	13 (6)	6 (6)	4 (6)		
NC123.10.A	25	21 (6)	0	0	0	0		
NC123.10.B	25	19 (6)	0	3 (6)	0	0		
NC123.10.C	25	21 (6)	0	11 (6)	0	0		
NC123.10.D	25	17 (6)	18 (6)	1 (6)	0	12 (7)		
NC15B	20	8 (6)		0	5 (6)		17 (7)	0
NC15B.4.A	25	4 (6)		0	23 (6)		0	20 (6)
NC15B.4.B	25	17 (6)		20 (6)	22 (6)		9 (7)	21 (6)
NC15B.4.C	25	22 (6)		12 (6)	21 (6)		23 (7)	21 (6)
NC15B.4.D	25	19 (6)		3 (6)	22 (6)		19 (7)	22 (6)

Markers are as described in Table 1. Markers K14, 6802, M4, AP4, and AP19 showed no changes.

*Number of lines homozygous for the P7076 (7) and the P6497 alleles (6).

†One single zoospore line from each F_1 progeny was selected (e.g. NC123.10), and four independent subcultures (A–D) were established from it (see *Materials and Methods*).

GENETICS

during subsequent growth of the oospore germlings, we selfed selected single zoospore isolates and reisolated zoospores from F_2 progeny heterozygous for the test markers. We selfed the same three single zoospore isolates that we had tested in the previous section, namely NC174.6, NC123.10, and NC15B.4. For lines NC123.10 and NC15B.4, the average conversion frequency was increased substantially compared with the original F_1 lines, NC123 and NC15B (1.8- and 2.7-fold, respectively; $P < 0.001$ in both cases), or with the vegetatively passaged lines (2.2- and 1.25-fold, respectively; $P < 0.001$ in both cases). Line NC174.6 showed a significant increase relative to the vegetatively passaged lines (1.67-fold; $P < 0.005$) but not relative to the original F_1 line.

Discussion

We have shown that changes to homozygosity occur at high frequency during vegetative growth of certain *P. sojae* hybrids, especially those involving strain P7076, as a result of gene conversion without crossing over. Conversions occurred not only within the linkage group we selected for study but at four of five randomly chosen unlinked markers. Distorted segregation, consistent with high frequency gene conversion, also was observed at a further five unlinked restriction fragment length polymorphism markers (21). Thus high frequency mitotic gene conversion seems widespread across the *P. sojae* genome. In *P. sojae*, which is diploid, haploid nuclei in the gametangia are formed by meiosis and do not undergo division (2). The genomes of the two parents cannot interact in the F_1 progeny until after the gametangia fuse. Therefore the changes to homozygosity cannot occur during meiosis.

The sizes of the conversion tracts appear to be less than 1 kb, because no evidence for coconversion was observed among three loci spaced 2.7 and 1.3 kb apart (4141, 6802, and 8127, respectively). Although 8 of 33 zoospore lines from NC123 showed conversion at both 8127 and 8801 (674 bp apart), 12 zoospore lines from this F_1 did not. Moreover, of 62 zoospore lines from seven other F_1 that showed conversion for either 8801 or 8127, none showed conversion of both loci. Thus the simultaneous conversion of 8127 and 8801 in some derivatives of NC123 is likely to have occurred by independent events, and the conversion tracts in these cases therefore are likely to have been less than 700 bp. In *Saccharomyces cerevisiae*, mitotic gene conversion tracts ranged from 50 to 400 bp in length (38, 39).

Mechanism of Gene Conversion Disparity. Of the 12 loci with codominant markers that underwent conversion, six of the loci (K14, 21J17L, 8127, 4141,19N3R, and 4N8R) showed disparity as to which allele was lost. For example, 242 zoospore lines (from 20 F_1 and F_2 individuals) showed conversion at 19N3R (Tables 1 and 4 and data not shown); in every case conversion favored the allele from P7076. In all cases except K14, the number of conversions was high enough to rule out that all of the conversions occurred in favor of the same allele by chance. One possible trivial explanation for the observed disparity is that the favored allele in each case is actually duplicated in the genome, so that loss of that allele caused by conversion is masked. This can be ruled out fully for loci 4141 and 8127 where we have carried out extensive characterization of the region spanning these two loci by fine structure genetic mapping, DNA sequencing, and Southern blotting (in pursuit of the *Avr1b* gene; W.-x.S. and B.M.T., unpublished data). Similar but less comprehensive analyses of the regions spanning 21J17L, 19N3R, and 4N8R also make it unlikely that alleles at these loci are duplicated.

Strong disparity is characteristic of gene conversions initiated by double-stranded chromosome breaks at specific DNA sequences, the best example of which is mating type switching in *S. cerevisiae* (40). Gene conversion without disparity can be initiated this way if double-stranded breaks can occur on DNA strands carrying either allele. Single-stranded breaks also could account for gene conversion without disparity. We speculate that single- or double-stranded DNA cleavage near converted markers is stimulated when the genomes of P6497 and P7076 come together. In some sense therefore, the two genomes seem incompatible. One possible mechanism could be the presence of different bacterial-style restriction-modification systems (41) in the two strains. In this case, a nuclease encoded by one genome may cleave DNA from the other genome before it becomes fully modified by the methylase (or other enzyme) encoded by the first genome. Another possibility is that cleavage is caused by transposases encoded by transposons that are silent normally but are activated after mating (similar to transposon dysgenesis in *Drosophila*, reviewed in ref. 42) A third possibility is that site-specific nucleases may be activated transiently as the result of nonself recognition after mating. This mechanism would be similar to vegetative incompatibility that occurs in true fungi when two strains that contain specific nonself recognition genes fuse, resulting in programmed cell death of the fused hyphae (43). The disparity observed for conversion of a particular marker could result from the absence of the recognition or cleavage site from one allele (the favored allele) or differential modification of the site in the two different genomes.

The Initiation of Gene Conversion May be a Two-Step Process. Pedigree analysis showed that gene conversions at individual loci occur more than once in clones of individual F_1 progeny. This result is consistent with the very high conversion frequencies observed at some loci. However, it is not consistent with the observation that in the case of loci that undergo conversions in favor of either allele, all conversions favor one allele in a given F_1 or F_2 individual. For example, six F_1 individuals show gene conversion at locus 8801. Among these, all conversions in the F_1 individuals NC160, NC16, and NC67 are in favor of the P6497 allele, whereas all those in the F_1 individuals NC123, NC15A, and NC15B are in favor of the P7076 allele. These results would be expected if each gene conversion event (or a committed step toward it) occurred only once, early in the expansion of the clone. Similarly, for several markers (e.g., 21J17L and 4N8R) changes occurred at a high frequency but only among the zoospore lines of a small number of F_1 individuals. This observation suggests that for these markers, gene conversion is not very common, because most F_1 individuals do not have the change, and that the F_1 individuals that do show the change have a high frequency of the change among their zoospores, because a single change occurred early in the growth of the F_1.

To reconcile the data that suggests that conversions occur frequently with those that suggest the frequency is low, we propose that in *P. sojae* mitotic gene conversion is initiated by a two-step process. The first step usually occurs early in the growth of an F_1 and, for many markers, at relatively low frequency. This step "activates" or predisposes the F_1 subsequently to undergo high frequency changes at a particular site. It also commits which allele will be lost if conversion occurs but does *not* commit the nucleus to complete the conversions. The second step is the completion of gene conversion, which occurs many times in different nuclei as a result of the activation but only in a percentage of activated nuclei. The first step is responsible for the observation that several of the markers show a high frequency of changes, but only among the zoospore lines of a small number of F_1 individuals. It also explains why all conversions in a clone occur in favor of the same allele. The second step is responsible for the randomization of the order of changes.

Several possible mechanisms can be imagined for steps one and two. An essential feature of the activation must be that the activated state can be propagated through nuclear division such that a population of nuclei is created in which actual conversion of the markers occurs in many different orders or may not occur at all in some nuclei. One possibility is that step one is a chemical modification of a DNA recognition or cleavage site, for example by the introduction of a nick (as in *Schizosaccharomyces pombe* mating type switching; ref. 44) or by the addition or removal of methyl groups (as in bacterial-style restriction-modification systems). Alteration of chromatin configuration similar to position effects on

transcription (42, 45) is a third possibility. Step two then would be cleavage by an enzyme recognizing the modification state, leading to gene conversion. Alternatively, step one could be activation of a nuclease(s) that recognizes the DNA cleavage sites; step two would be actual cleavage of the target sites.

Contribution of Gene Conversion to Genetic Diversity. When the *P. sojae* strains P6497 and P7076 were crossed, we observed high frequency mitotic gene conversion in the F_1 progeny, resulting rapidly in a heterokaryotic strain containing a highly diverse population of diploid nuclei. Even loci that showed close linkage during meiotic recombination were reassorted at high frequency. In the field, this mechanism would result in a large reservoir of diversity within an individual thallus that could facilitate a strain to adapt to new environmental challenges. The region we studied most closely contains a gene, *Avr1b* (W.-x.S. and B.M.T., unpublished data), that is subjected directly to selection pressure by specific resistance genes in the host plant. Changes to homozygosity were observed also in the region surrounding *Avr1a* (37), which is unlinked to *Avr1b* (21, 46). We showed previously that outcrosses between different *P. sojae* genotypes in the field are not common but have been a major source of new races of *P. sojae* (28) able to overcome new combinations of resistance genes in soybean cultivars. Mitotic gene conversion could well have facilitated the reassortment of avirulence genes that resulted in these new races, especially in the case of closely linked avirulence genes (46, 47). Because mitotic gene conversion was observed at many unlinked loci spread across the *P. sojae* genome, gene conversion may be responsible for creating diversity in all aspects of *P. sojae* growth, development, and pathogenicity. We observed very different gene conversion frequencies in the two crosses we analyzed, P6497 $\times$ P7076 and P6497 $\times$ P7064. The majority of the few *P. sojae* crosses reported in the literature (20, 21, 29) show approximately Mendelian segregation of alleles as in the cross of P6497 $\times$ P7064. Presumably, therefore, conversion frequencies comparable to P6497 $\times$ P7064 ($\approx 5 \times 10^{-5}$ per locus per nucleus per generation) are the norm in *P. sojae* unless crosses with severe distortion were not reported. Even the lower conversion rate would be likely to result in extensive variation given the extended periods of vegetative growth typical of this organism. The results from the P6497 $\times$ P7076 cross demonstrate that the machinery exists in this species for generating extremely high frequencies of gene conversion. Although this mechanism seems to operate constitutively in hybrids from this cross, it is possible that high frequency conversion is facultative in other strains of *P. sojae*. For example it might be induced by particular physiological conditions such as stress. If double-stranded breaks underlie the gene conversions we have observed here, then the breaks also may contribute to the genetic diversity of the pathogen by stimulating point mutations. In yeast, repair of double-stranded breaks increases the frequency of point mutations in the vicinity by 100-fold (48). Furthermore, in mammalian Ig genes expressed in antibody-producing cells, high frequency double-stranded breaks targeted to the variable regions of the genes promote somatic diversification of the genes by hypermutation as well as by gene conversion from neighboring pseudogenes (49).

Mitotic gene conversion also may operate in other oomycetes; a loss of heterozygosity was observed after extended vegetative culture of F_1 hybrids of *Pythium ultimum* (50), although closely linked markers were not examined to determine the mechanism. Nonstoichiometric ratios of alleles and mitotic instability have been reported in F_1 hybrids of *Phytophthora parasitica* (51) *P. infestans* (52), and *Phytophthora cinnamomi* (53). In the case of the latter two species, inheritance of three alleles in some hybrids suggested that abnormal chromosome segregation during meiosis was involved in some cases. However, because closely linked markers were not examined, gene conversion may have made an additional undetected contribution to the abnormal segregation. In principle, mitotic gene conversion could promote somatic or germline diversity in any diploid organism in which a period of nuclear or cellular division follows mating as it does here and in the case of mammalian antibody genes.

We thank Minh Cao and Dan Leung for identification of RAPD markers linked to *Avr1b* and Felipe Arredondo for technical assistance. This work was supported in part by United Soybean Board Grant 8210 and University of California Systemwide Biotechnology Program Grant 98-04. J.C. acknowledges support from a Royal Thai Government Fellowship.

1. Day, P. (1974) *Genetics of Host-Parasite Interactions* (Freeman, San Francisco).
2. Erwin, D. C. & Ribiero, O. K. (1996) *Phytophthora Diseases Worldwide* (APS, St. Paul, MN).
3. Shaw, D. S. (1983) in *Phytophthora: Its Biology, Taxonomy, Ecology and Pathology*, eds. Erwin, D. C., Bartnicki-Garcia, S. & Tsao, P. H. (American Phytopathological Society, St. Paul, MN) pp. 81–94.
4. Valent, B. & Chumley, F. G. (1991) *Annu. Rev. Phytopathol.* **29,** 443–467.
5. Miao, V. P., Covert, S. F. & Van Etten, H. D. (1991) *Science* **254,** 1773–1776.
6. Mills, D. & McCluskey, K. (1990) *Mol. Plant–Microbe Interact.* **3,** 351–357.
7. Henderson, I. R., Owen, P. & Nataro, J. P. (1999) *Mol. Microbiol.* **33,** 919–932.
8. Rudenko, G., Cross, M. & Borst, P. (1998) *Trends Microbiol.* **6,** 113–116.
9. Ji, C., Smith-Becker, J. & Keen, N. T. (1998) *Curr. Opin. Biotechnol.* **9,** 202–207.
10. Lauge, R. & De Wit, P. J. G. M. (1998) *Fungal Genet. Biol.* **24,** 285–297.
11. Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S. & Knogge, W. (1995) *EMBO J.* **14,** 4168–4177.
12. Orbach, M. J., Farrall, L., Sweigard, J. A., Chumley, F. G. & Valent, B. (2000) *Plant Cell* **12,** 2019–2032.
13. Jia, Y., McAdams, S. A., Bryan, G. T., Hershey, H. P. & Valent, B. (2000) *EMBO J.* **19,** 4004–4014.
14. Joosten, M. H. A. J., Cozijnsen, T. J. & de Wit, P. J. G. M. (1994) *Nature (London)* **367,** 384–386.
15. Keen, N. T., Tamaki, S., Kobayashi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D. & Staskawicz, B. (1990) *Mol. Plant–Microbe Interact.* **3,** 112–121.
16. Kearney, B. & Staskawicz, B. J. (1990) *J. Bacteriol.* **172,** 143–148.
17. Kamoun, S., Klucher, K. M., Coffey, M. D. & Tyler, B. M. (1993) *Mol. Plant–Microbe Interact.* **6,** 573–581.
18. Förster, H., Coffey, M. D., Elwood, H. & Sogin, M. L. (1990) *Mycologia* **82,** 306–312.
19. Gijzen, M., Macgregor, T., Bhattacharyya, M. & Buzzell, R. (1996) *Physiol. Mol. Plant Pathol.* **48,** 209–215.
20. Whisson, S. C., Drenth, A., Maclean, D. J. & Irwin, J. A. G. (1994) *Curr. Genet.* **27,** 77–82.
21. Tyler, B. M., Förster, H. & Coffey, M. D. (1995) *Mol. Plant–Microbe Interact.* **8,** 515–523.
22. Denward, T. (1970) *Hereditas* **66,** 35–48.
23. Fry, W. E. (1982) *Principles of Plant Disease Management* (Academic, New York).
24. Bruin, G. C. A. & Edington, L. V. (1981) *Can. J. Plant Pathol.* **3,** 201–206.
25. Crute, I. R. (1987) *Plant Dis.* **71,** 763–767.
26. Dowley, L. J. & O'Sullivan, E. (1981) *Potato Res.* **24,** 417–421.
27. Rutherford, F. S., Ward, E. W. B. & Buzzell, R. I. (1985) *Phytopathology* **75,** 371–374.
28. Förster, H., Tyler, B. M. & Coffey, M. D. (1994) *Mol. Plant–Microbe Interact.* **7,** 780–791.
29. Bhat, R. G. & Schmitthenner, A. F. (1993) *Exp. Mycol.* **17,** 122–129.
30. Long, M. & Keen, N. T. (1977) *Phytopathology* **67,** 675–677.
31. Layton, A., C. & Kuhn, D. N. (1990) *Phytopathology* **80,** 602–606.
32. Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. (1990) *Nucleic Acids Res.* **18,** 6531–6535.
33. Tyler, B. M., Wu, M.-H., Wang, J.-M., Cheung, W. W. S. & Morris, P. F. (1996) *Appl. Environ. Microbiol.* **62,** 2811–2817.
34. Nataraj, A. J., Olivos-Glander, I., Kusukawa, N. & Highsmith, W. E. (1999) *Electrophoresis* **20,** 1177–1185.
35. Esposito, M. S., Ramirez, R. M. & Bruschi, C. V. (1994) *Curr. Genet.* **26,** 302–307.
36. Tsang, P. W. K., Cao, B., Siu, P. Y. L. & Wang, J. (1999) *Microbiology* **145,** 1623–1629.
37. Macgregor, T. (2001) Ph. D. thesis (University of Western Ontario, London, ON, Canada).
38. Sweetser, D. B., Hough, H., Whelden, J. F., Arbuckle, M. & Nickoloff, J. A. (1994) *Mol. Cell. Biol.* **14,** 3863–3875.
39. Nickoloff, J. A., Sweetser, D. B., Clikeman, J. A., Khalsa, G. J. & Wheeler, S. L. (1999) *Genetics* **153,** 665–679.
40. Herskowitz, I., Rine, J. & Strathern, J. (1992) in *The Molecular and Cellular Biology of the Yeast Saccharomyces*, eds. Broach, J. R., Pringle, J. R. & Jones, E. W. (Cold Spring Harbor Labor. Press, Plainview, NY) pp. 583–656.
41. Redaschi, N. & Bickle, T. A. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Curtiss, R., Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC) pp. 773–781.
42. Fritze, C. E., Verschueren, K., Strich, R. & Esposito, R. E. (1997) *EMBO J.* **16,** 6495–6509.
43. Leslie, J. F. & Zeller, K. A. (1996) *J. Genet.* **75,** 415–424.
44. Arcangioli, B. (1998) *EMBO J.* **17,** 4503–4510.
45. Stavenhagen, J. B. & Zakian, V. A. (1998) *Genes Dev.* **12,** 3044–3058.
46. Whisson, S. C., Drenth, A., Maclean, D. J. & Irwin, J. A. G. (1995) *Mol. Plant–Microbe Interact.* **8,** 988–995.
47. Gijzen, M., Forster, H., Coffey, M. D. & Tyler, B. M. (1996) *Can. J. Bot.* **74,** 800–802.
48. Strathern, J. N., Shafer, B. K. & McGill, C. B. (1995) *Genetics* **140,** 965–972.
49. Sale, J. E., Calandrini, D. M., Takata, M., Takeda, S. & Neuberger, M. S. (2001) *Nature (London)* **412,** 921–926.
50. Francis, D. M., Gehlen, M. F. & St Clair, D. A. (1994) *Mol. Plant–Microbe Interact.* **7,** 766–775.
51. Förster, H. & Coffey, M. D. (1990) *Exp. Mycol.* **14,** 351–359.
52. Carter, D. A., Buck, K. W., Archer, S. A., Van der Lee, T., Shattock, R. C. & Shaw, D. S. (1999) *Fungal Genet. Biol.* **26,** 198–208.
53. Dobrowolski, M. P., Tommerup, I. C., Blakeman, H. D. & O'Brien, P. A. (2001) *Fungal Genet. Biol.*, in press.
54. Lamb, B. C. (1998) *Heredity* **80,** 538–552.