Isozyme Analysis to Differentiate Species of *Peronosclerospora* Causing Downy Mildews of Maize

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ABSTRACT

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Isozymes from 10 fungal cultures representing three species of the genus *Peronosclerospora* were compared electrophoretically on starch gels to determine if isozyme analysis can be used to aid in species identification. Cultures of *P. sorghi* from Texas (United States), southern India, and Brazil were nearly identical with apparent polymorphisms (allelic variations) at three of 13 presumed loci examined. Of 15 apparent alleles detected in an isolate of *P. sorghi* from Thailand, only four were in common with alleles in any of five other cultures of typical *P. sorghi*. Two cultures of *P. philippinensis* from the Philippines and one of *P. sacchari* from Taiwan had all 16 alleles in common indicating that they are most likely of the same species. In four cultures of the *P. sacchari–P. philippinensis* complex, apparent polymorphisms were evident in six of 13 loci examined. Considerable potential for variation exists in this "species." The isolate of *P. sorghi* from Thailand was more closely related to the *P. sacchari–P. philippinensis* complex than to the other isolates of *P. sorghi*. An average coefficient of similarity of 0.50 was estimated by comparing the Thailand culture with *P. sacchari* and *P. philippinensis* at 13 loci. Isozyme analysis is useful for distinguishing species of *Peronosclerospora* and elucidating phylogenetic relationships.

Additional key words: systematics, isozyme electrophoresis, genetics.

The *Peronosclerospora* spp. that cause downy mildew of maize and other gramineous hosts include some of the most destructive pathogens in the tropics and subtropics (5), and some of them infect more than one important crop. Of the 10 recognized species of fungi that cause downy mildew diseases on maize, five currently are in the newly erected genus *Peronosclerospora* (25,27).

The confusion over nomenclature of specific pathogens involved in these diseases is a major problem in global evaluation of the downy mildews caused by *Peronosclerospora*. It is difficult to determine if research results and disease control methods developed in one area of the world might be applicable to another. In several instances, taxonomy within the genus is confused because, at most, only slight differences in morphology of recognized species exist (23). Furthermore, extensive morphological variation exists within the species. Exconde et al (4) observed differences in size and shape of conidiophores and conidia after inoculating various hosts with *Peronosclerospora philippinensis* (Weston) C. G. Shaw. Leu (9) in Taiwan determined that temperature during sporulation and host cultivar affected size and shape of conidia of *P. sacchari* (T. Miyake) Shirai and Hara. Pupipat (16) was able to classify 35 isolates of *P. sorghi* (Weston and Uppal) C. G. Shaw on maize in Thailand into 10 categories based on conidial shapes that ranged from globose to dumbbell to cylindrical.

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Electrophoretic analysis of isozymes has been used extensively and successfully for years to provide rapid, quantitative estimates of the extent of genic variation within species of animals (15) and higher plants (30), and more recently for nematodes (8). Because amino acid sequences of polypeptides (components of enzymes) are dependent on nucleotide sequences of their coding genes, an analysis of isozyme variation by electrophoresis approximates the analysis of genic variation (10). The ambiguous results of early isozyme analysis in fungi (26) discouraged application of this methodology; however, fungi have been used recently in studies to quantify genic variation. For example, in 1975 Spieth (28) successfully quantified genic variation at specific loci in populations of *Neurospora intermedia* Tai. May et al (11) examined intraspecific genetic variability in laboratory strains of *Entomophthora* by means of electrophoresis of isozymes, and Royse and May (19,20) and May and Royse (12) used isozyme analysis to quantify variation in the common edible mushroom, *Agaricus brunnescens* Peck, to identify genotypic classes within the species, and to confirm crosses between lines of the mushroom. In a few instances, isozymes have been used in an attempt to distinguish species of fungi; however, differences were based on the presence or absence of bands appearing on the gels (eg, 22,24,29).

The purpose of our study was to compare isozymes of several isolates of *Peronosclerospora* representing three recognized species, and to evaluate isozyme analysis as a method for identifying species within this genus. Previous studies showed that cultures of two of the species, *P. sacchari* and *P. philippinensis*, were nearly identical in several aspects, including their host ranges, responses to environmental conditions, and morphology of conidia and conidiophores (2). If isozyme comparison can be used to distinguish species of *Peronosclerospora*, it will be a valuable tool for providing a more reliable means of identification. Because of the far greater amount of information that can be obtained by interpreting isozyme banding patterns based on genetic interpretations, rather than by band counting, we have adopted the genetic interpretation approach in our study.

MATERIALS AND METHODS

Cultures. All cultures used were field collections potentially derived from many spores of the pathogen. *P. sacchari* was obtained in 1975 and 1983 from infected sugarcane sets sent to PDRL from Taiwan. Infection in maize was initiated from conidia collected from sugarcane plants grown from the sets. Later, a subculture of the 1975 culture was established from conidia of a single conidiophore and used for electrophoretic studies.

Cultures of *P. philippinensis* were obtained in 1975 and 1979 from infected maize plants in Los Baños, the Philippines, and an isolate of *P. sorghi* was obtained from infected maize in Thailand. Cultures of *P. sorghi* from Texas (Tx), United States; Mysore, India (In); and Jaboticabal S.P., Brazil (Bz) were collected as oospores from infected sorghum. Infection in maize was later established at PDRL from oospore inoculations. In this manuscript, because the Thailand culture of *P. sorghi* behaves very differently from all other cultures of *P. sorghi*, for the purpose of clarity it is referred to as the atypical *P. sorghi*. All other cultures of *P. sorghi* are referred to as typical cultures of *P. sorghi*. All cultures were maintained on maize (*Zea mays* L. 'DeKalb XL55a' or 'Pioneer 3369A').

Production and treatment of conidia for electrophoresis. Each culture was inoculated on about 60 plants (two plants per 10-cm-diameter clay pot) at the two-leaf stage. An atomizer with 3,500 $kg \cdot m^{-2}$ (5 $lb \cdot in.^{-2}$) air-line pressure was used to apply an inoculum suspension which contained 5.0×10^3–1.0×10^4 conidia per milliliter. Two isolates of *P. sorghi* also were used to infect grain sorghum [*Sorghum bicolor* (L.) Moench 'Tophand'] by spraying seedlings at the two-leaf stage with suspensions containing 1.0×10^4 spores per milliliter, 1 ml per pot of seedlings. A suspension of 5.0×10^4 conidia of *P. sacchari* per milliliter was used to infect sugarcane (*Saccharum officinarum* L. 'CP-44-101') by injecting 2 ml of the suspension into the base of emerging shoots of the sugarcane sets. Following inoculation, all plants were incubated overnight in dew chambers at 20 C and then placed in the greenhouse or a growth chamber with a 12-hr day length and 26/15 C (day/night) temperatures for disease development. The intentional lack of dew formation prevented sporulation until systemically infected plants were placed in dew chambers. In addition, most cultures were also maintained in separate greenhouse rooms with separate filtered-air supplies to ensure that mixing did not occur.

At the appropriate stage of infection for each culture-host combination, systemically infected plants were exposed to supplemental light from Sylvania 1,000-W Metalarc high-intensity lamps for about 16 hr (from 1600 to 0800 hours) and then placed in dark dew chambers at 20 C for 5.0–7.5 hr (time depending on the pathogen culture and particular experiment) to induce both dew formation and sporulation. Conidia were collected by washing spores from leaves with a fine spray of cold (5 C) distilled water delivered by an atomizer at about 3,500 $kg \cdot m^{-2}$ (5 $lb \cdot in.^{-2}$) air-line pressure. The spore suspension immediately was filtered through a 150-μm (100-mesh) screen. Similar numbers of noninfected maize and sorghum plants the same age as the infected plants were sprayed with water, and leaf washings were used as controls.

Approximately 15 ml of conidial suspensions or control washings were centrifuged at 700 *g* relative centrifugal force for 5–15 min. After centrifugation, most of the water was removed by pipette, leaving 1 ml containing a pellet. The pellet was resuspended in the remaining water and transferred to a 2-ml 'Nunc' round bottom cryotube (Arthur H. Thomas Co., Philadelphia, PA) which was stored in liquid nitrogen until used.

Tubes containing the frozen samples were transported in liquid nitrogen to Ithaca, NY.

Each frozen sample was crushed with an 8-mm-diameter glass rod, the end of which had been melted to form a bulb which fit snugly into the 'Nunc' vial. Prior to crushing a sample, the rod was submerged into liquid nitrogen. Crushing continued until the sample completely thawed. About 40–60% of the conidia were ruptured. The crushed samples were transferred to 12 × 75-mm disposable test tubes, brought to 10.5 ml with 0.05 M tris HCl buffer, pH 7.1, and centrifuged to remove solid debris.

Gel electrophoresis. Horizontal starch gel electrophoresis was performed according to the procedures described by May et al (14). Staining techniques were those of Allendorf et al (1) except for AAT (see Table 1 for enzyme abbreviations and buffers). Staining for AAT was after May et al (14).

Genic nomenclature. The genic nomenclature of May et al (14) was used. Capital-lettered abbreviations referred to enzymes (Table 1), whereas abbreviations with only the first letter capitalized referred to putative loci coding for the enzyme. Alleles at a particular locus were designated by the relative anodal or cathodal mobility from the origin of their homomeric (all polypeptide components the same) protein products. The designation for each allele was relative to the homomeric protein product of one allele (usually the most common) designated 100. For example, the dimeric enzyme Gpi (Table 1), would stain on a starch gel in a three-banded pattern. A culture with Gpi staining at 100 and 130 would be heterozygous (or heterokaryotic if N+N) for alleles 130 and 100 and would be designated 130/100. A culture designated Gpi (130/130) would be homozygous at the Gpi locus for allele 130 and would appear on a starch gel as a single band.

Interpretation of results. Although cultures were not derived from single spores, the results were not complex and were interpretable. This allowed isolates of a given species to be compared by means of the coefficient of similarity (CS) of Rogers (18), a method of comparing isolates of the same species for degree of genic similarity at specific loci.

Examination of banding patterns among species suggested that for most, if not all, enzymes the same loci were involved. This is not surprising since these downy mildew pathogens of maize all have many characteristics in common and possibly could be considered the same species. By making the assumption that the same loci were common to each pathogen, coefficients of similarity were calculated to compare cultures across species. The cultures were also compared in terms of the number of presumed alleles in common.

RESULTS AND DISCUSSION

Table 1 lists enzymes for which activity was found and the buffer used for resolution. Results for each enzyme were not influenced by host species on which the pathogen was grown, the time of year of conidial collection prior to freezing, or by whether infected plants were grown in the greenhouse or in a growth chamber. Non-germinated spores gave slightly more prominent bands than spores that had been allowed to germinate for 0.5 hr prior to freezing, indicating higher enzyme levels in the ungerminated spores. Enzymes were not detected in the control leaf washings indicating that the enzymes likely were not of host origin. Best results were obtained when at least 1.5×10^7 spores were present in the sample for electrophoresis. Results for specific enzymes are depicted in Fig. 1 and Table 2.

Glucosephosphate isomerase (GPI). *P. sorghi* Tx '72 (phenotype 1) was homozygous for allele Gpi 100, whereas all other isolates of typical *P. sorghi* (phenotype 2) were heterozygous for alleles (Gpi 100 and Gpi 130 (Fig. 1, Table 2). *P. sacchari* '75, *P. philippinensis* '75, and *P. philippinensis* '79 (phenotype 3) were identical and homozygous for Gpi 153. *P. sorghi* Thai (phenotype 4) was homozygous for Gpi 180 and *P. sacchari* '83 (phenotype 5) was homozygous for Gpi 207.

Malate dehydrogenase (MDH). *P. sorghi* Thai (phenotype 1) produced a single band that moved anodally and the culture apparently was homozygous for allele Mdh-43. All typical isolates of *P. sorghi* exhibited phenotype 2, homozygous for allele Mdh 57. *P. sacchari* '75, *P. philippinensis* '75, and *P. philippinensis* '79 exhibited phenotype 3, homozygous for allele Mdh 100, and *P. sacchari* '83 produced phenotype 4, homozygous for Mdh 107.

TABLE 1. List of enzymes for which activity was found, abbreviations, subunit compositions of enzymes, and buffer systems

Name	Abbreviation	Subunit composition	Buffer
Aspartate aminotransferase	AAT	dimer[a]	C[b]
Fumarase	FUM	tetramer	C
Glucosephosphate isomerase	GPI	dimer	C
Glutamate pyruvate transaminase	GPT	dimer	M[c]
Glutathione reductase	GR	dimer	M
Isocitrate dehydrogenase	IDH	dimer	C
Leucine aminopeptidase	LAP	monomer	M
Malate dehydrogenase	MDH	dimer	C
Peptidase with glycyl-leucine	PEP-GL	···	R[d]
Peptidase with leucyl-leucyl-leucine	PEP-LLL	dimer	M
Phosphogluconate dehydrogenase (6-Phosphogluconate dehydrogenase)	PGD	dimer	C
Superoxide dismutase	SOD	dimer	M

[a] Enzyme subunit compositions are according to Harris and Hopkinson (7).
[b] Electrode buffer = 0.04 M citric acid adjusted to pH 6.1 with *N*-(3-aminopropyl)-morpholine, diluted 1:10 for gel buffer according to Clayton and Tretiak (3); ≤ 90 mA, 200 V.
[c] Electrode buffer according to Markert and Faulhaber (10); ≤275 V, ≤75 mA; electrode buffer diluted 1:5 for gel.
[d] Buffer according to Ridgway et al (17); ≤250 V, ≤75 mA.

Aspartate aminotransferase (AAT). Except for the Thai isolate, all isolates of *P. sorghi* exhibited phenotype 1, homozygous for Aat 94. *P. sacchari* '75, *P. philippinensis* '75, *P. philippinensis* '79, and *P. sorghi* Thai exhibited phenotype 2, apparently heterozygous for alleles Aat 100 and Aat 81. *P. sacchari* '83 (phenotype 3) was homozygous for Aat 100. That phenotype 2 did not produce the 1:2:1 ratio of band intensities characteristic for a dimeric enzyme coded by heterozygous alleles could be due to the fact that the isolates did not originate from single spores and represent mixtures of genotypes. It is also possible that the subunits of the heteromer do not combine at random in the cytoplasm.

Isocitrate dehydrogenase (IDH). All typical isolates of *P. sorghi* produced phenotype 1, homozygous for allele Idh 162. *P. sorghi* Thai produced phenotype 2, homozygous for Idh 133. *P. sacchari* '75, *P. philippinensis* '75, and *P. philippinensis* '79 exhibited phenotype 3, heterozygous for Idh 100 and Idh 76, and *P. sacchari* '83 exhibited phenotype 4, heterozygous for Idh 90 and Idh 67. The unsymmetrical banding patterns for phenotypes 3 and 4 suggest that the enzyme subunits were combining in a nonrandom manner.

Superoxide dismutase (SOD). The five typical isolates of *P. sorghi* and *P. sacchari* '83 produced phenotype 1, homozygous for Sod 111. *P. sorghi* Thai, *P. sacchari* '75, *P. philippinensis* '75, and *P. philippinensis* '79 exhibited phenotype 2, homozygous for Sod 100.

Peptidase with glycyl-leucine (PEP-GL). *P. sacchari* '83 and all typical isolates of *P. sorghi*, except *P. sorghi* Bz (no enzyme detected), produced phenotype 1, homozygous for allele 107. Probably the enzyme did not show up with the Brazilian isolate because of the low numbers of spores collected. *P. sacchari* '75, the two isolates of *P. philippinensis*, and *P. sorghi* Thai all gave phenotype 2, homozygous for Pep-GL 100.

Glutamic pyruvic transaminase (GPT). All isolates of *P. sorghi*, including that from Thailand, produced phenotype 1; all isolates of *P. sacchari* and *P. philippinensis* exhibited phenotype 2. Both phenotypes had two bands. It is doubtful that the presence of two bands for each of the 10 isolates is the result of two alleles at a single locus within the 10 populations, because one would expect some heterokaryotic individuals in at least some of the cultures, hence the presence of heteromeric bands. The presence of two bands, instead of one, for each isolate may be caused by any of several factors. One explanation is that with each isolate two loci code for GPT, and a second is that the enzyme exists in two transformational states coded by homozygous alleles. Presently, it is not possible to say which of these (or another explanation) is correct. For the purpose of the genetic comparisons made in this manuscript, we take the conservative approach and assume the presence of only a single locus with only one allele for each phenotype. The alleles are designated Gpt 100 and Gpt 110, respectively. All typical isolates of *P. sorghi* are homozygous for Gpt 100 and all other isolates are homozygous for Gpt 110.

Leucine aminopeptidase (LAP). All isolates of *P. sacchari* and *P. philippinensis*, in addition to *P. sorghi* Thai, produced phenotype 1, homozygous for Lap 112. All typical isolates of *P. sorghi* exhibited phenotype 2, homozygous for Lap 100.

TABLE 2. Presumed genotypes of 10 isolates of *Peronosclerospora* causing downy mildew on maize[a]

Genotypic class[b]	Loci											No. of isolates in class
	Gpi	Mdh	Aat	Idh	Sod	Pep-GL	Gpt	Lap	Gr	Fum	Pep-LLL-2	
1	130/100	57/57	94/94	162/162	111/111	107/107	100/100	100/100	96/96	85/85	100/100	2
2	130/100	57/57	94/94	162/162	111/111	107/107	100/100	100/100	100/100	100/100	100/100	1
3	130/100	57/57	94/94	162/162	111/111	0	100/100	100/100	96/96	85/85	100/100	1
4	100/100	57/57	94/94	162/162	111/111	107/107	100/100	100/100	96/96	85/85	100/100	1
5	153/153	100/100	100/81	100/76	100/100	100/100	110/110	112/112	109/109	137/137	109/109	3
6	207/207	107/107	100/100	90/67	111/111	107/107	110/110	112/112	109/109	137/137	109/109	1
7	180/180	−43/−43	100/81	133/133	100/100	100/100	100/100	112/112	100/100	106/106	109/109	1

[a] Two monomorphic loci common to all isolates also were detected.
[b] 1 = *P. sorghi* India 1982, and *P. sorghi* Texas 1981; 2 = *P. sorghi* India 1980; 3 = *P. sorghi* Brazil 1982; 4 = *P. sorghi* Texas 1972; 5 = *P. sacchari* 1975, *P. philippinensis* 1975, and *P. philippinensis* 1979; 6 = *P. sacchari* 1983; and 7 = *P. sorghi* Thailand 1976.

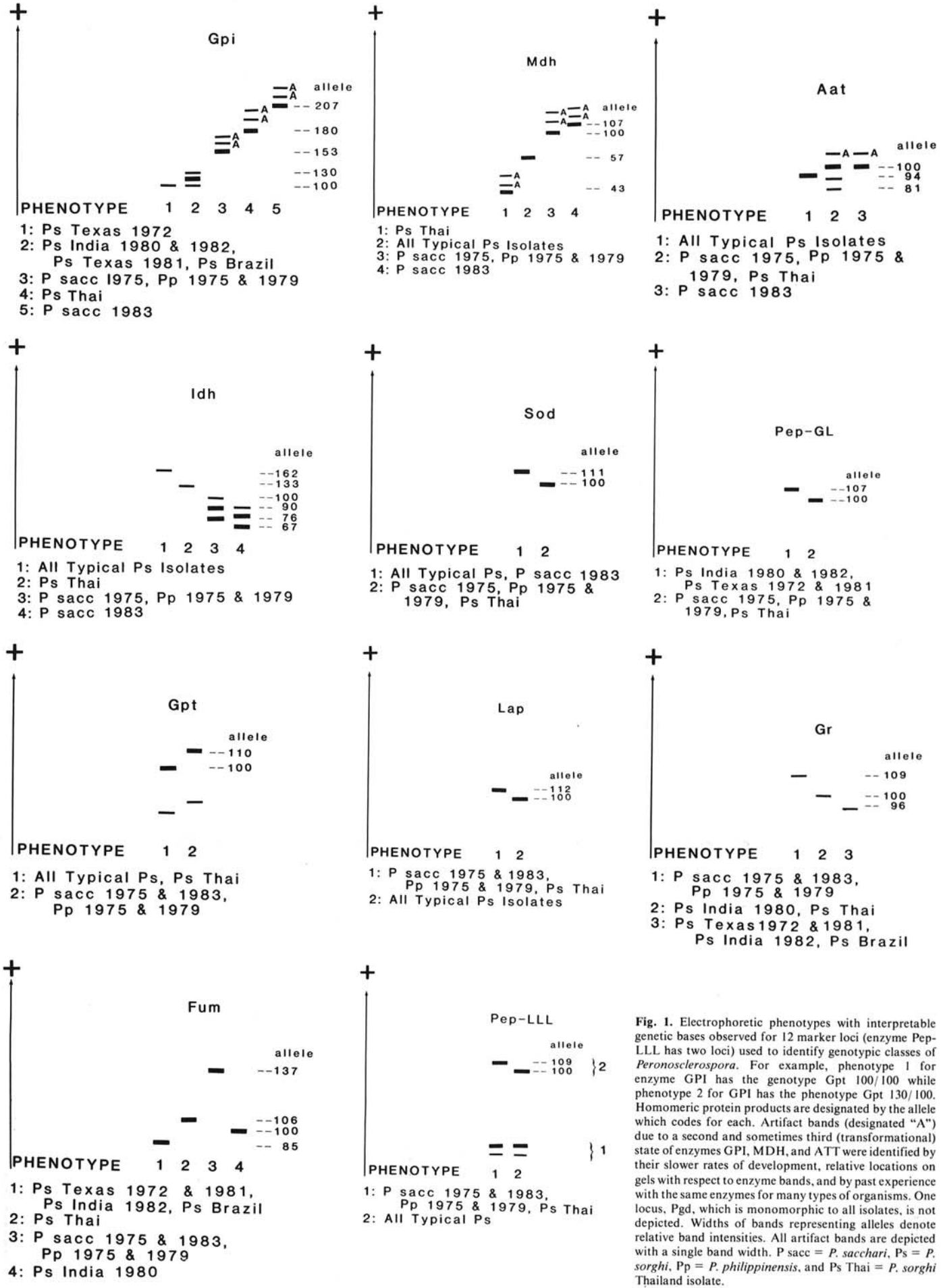

Fig. 1. Electrophoretic phenotypes with interpretable genetic bases observed for 12 marker loci (enzyme Pep-LLL has two loci) used to identify genotypic classes of *Peronosclerospora*. For example, phenotype 1 for enzyme GPI has the genotype Gpt 100/100 while phenotype 2 for GPI has the phenotype Gpt 130/100. Homomeric protein products are designated by the allele which codes for each. Artifact bands (designated "A") due to a second and sometimes third (transformational) state of enzymes GPI, MDH, and ATT were identified by their slower rates of development, relative locations on gels with respect to enzyme bands, and by past experience with the same enzymes for many types of organisms. One locus, Pgd, which is monomorphic to all isolates, is not depicted. Widths of bands representing alleles denote relative band intensities. All artifact bands are depicted with a single band width. P sacc = *P. sacchari*, Ps = *P. sorghi*, Pp = *P. philippinensis*, and Ps Thai = *P. sorghi* Thailand isolate.

Glutathione reductase (GR). All isolates of *P. sacchari* and *P. philippinensis* produced phenotype 1, homozygous for Gr 109. *P. sorghi* Thai and *P. sorghi* In '80 exhibited phenotype 2, homozygous for Gr 100. *P. sorghi* Tx '72, *P. sorghi* Tx '81, *P. sorghi* In '82, and *P. sorghi* Bz produced phenotype 3, homozygous for Gr 96.

Fumarase (FUM). *P. sorghi* Bz, *P. sorghi* Tx '72, *P. sorghi* Tx '81, and *P. sorghi* In '82 each exhibited phenotype 1, homozygous for Fum 85. *P. sorghi* Thai gave phenotype 2, homozygous for Fum 106, and the cultures of *P. sacchari* and *P. philippinensis* exhibited phenotype 3, homozygous for Fum 137. We consider that phenotypes 2 and 3 each are the result of a single allele but recognize that because the bands were beginning to resolve into multiple bands that the individuals may be heterozygous. *P. sorghi* In '80 produced phenotype 4, homozygous for Fum 100.

Peptidase with leucyl-leucyl leucine (PEP-LLL). *P. sorghi* Thai and all isolates of *P. sacchari* and *P. philippinensis* exhibited phenotype 1, whereas all typical isolates of *P. sorghi* exhibited phenotype 2. Both phenotypes presumably resulted from genes at two loci, Pep-LLL-1 (monomorphic) and Pep-LLL-2 (polymorphic). Locus Pep-LLL-2 produced bands at a higher zone on the gel than Pep-LLL-1. The bands in the upper zone developed at a different rate than bands in the lower zone. The existence of two zones of enzyme activity concomitant with different rates of development, and the constancy of the banding pattern in one zone but not the other all indicate the presence of two loci.

P. sorghi Thai and the isolates of *P. sacchari* and *P. philippinensis* (phenotype 1) were homozygous for Pep-LLL-2 allele 109 whereas all typical *P. sorghi* (phenotype 2) isolates were homozygous for Pep-LLL-2 allele 100.

The phenotype for Pep-LLL-1 probably is the result of one homozygous allele for all 10 isolates, even though two bands always were present. Although it is possible that another locus, common to all isolates, is involved, as with GPT, for comparative purposes we take the conservative approach and assume the presence of only one locus. The presence of two bands can be explained by the existence of two alleles at the Pep-LLL-1 locus for two segments of each population for each of the 10 cultures, but this situation is highly unlikely. Another, much more likely, explanation is that the enzyme has two transformational states.

General considerations. Electrophoretic separation of specific enzymes is an extremely useful tool that has several advantages over some of the more common methods for comparing organisms. First, it is relatively easy to obtain information on a large number of characters, eg, 10 or more enzymes. This is an advantage for organisms where phenotypic characters based on morphology or other characteristics are few, or differences are subtle or ambiguous, such as with many of the downy mildew pathogens. Another significant advantage is that comparisons generally are made with enzymes that are always present and influenced little by environmental factors (6). We found in our study that moderate environmental differences, such as different lighting conditions during different times of the year, or growth chamber versus greenhouse conditions, did not affect isozyme patterns. Finally, analysis of isozyme variation allows a quantitative interpretation of differences on the basis of genetics. Much more information can be obtained by isozyme analysis with a genetic basis than by other electrophoretic techniques where comparisons focus on the presence or absence, or intensity differences, of single bands, often with no information on the genetic basis of the proteins producing the bands.

Each culture we used was derived from a population of spores, although one culture, *P. sacchari* '75, was reestablished from conidia of a single conidiophore. To date, we have not been successful in obtaining infection from single spores. The inability to obtain single-spored lines need not prevent isozyme analysis based on genetics (11,28).

It was not known whether the asexual stage, including conidia, was diploid (2N), dikaryotic (N+N), or haploid (N). However, the results of a cytological study by Safeeulla and Thirumalachar (21) describing development of antheridia, oogonia, fusion nuclei, and mature oospores of *P. sorghi* indicate that two genomes are present in the mycelium in infected plants and presumably also in conidia. The formation of heteromeric bands in our study demonstrated that the asexual stage must be either diploid or dikaryotic, since heteromeric bands were produced and could only form with alleles present in the same thallus.

Although the number of isolates was small, polymorphic loci for typical *P. sorghi* were detected for GPI, GR, and FUM (Fig. 1, Table 2). Even though GR and FUM did not display heteromeric bands for typical *P. sorghi* (all isolates were homozygous), it was concluded that both GR and FUM each were coded for by the same locus present in all isolates of typical *P. sorghi*. Experience of numerous isozyme workers (eg, 1,13,32,33) has shown that bands produced by the same locus tend to be in the same zone on the gel and develop at the same rate. Furthermore, strong evidence that the alleles Gr 100 and Gr 96 (or Fum 85 and Fum 100) are at the same locus is that only one band developed for each phenotype. In normal evolutionary development, a new locus generally does not appear concomitantly with loss of the original locus.

Isolates *P. sacchari* '75, *P. philippinensis* '75, and *P. philippinensis* '79 each produced the same phenotypes for all 12 enzymes detected in the study (Fig. 1, Table 2). This is consistent with previous host-range, morphology, and response-to-environment data (2) suggesting that the isolates are very closely related and indeed probably are of the same species. Weston (31), when he described *P. philippinensis* in 1920 as a new species, stated that *P. sacchari* and *P. philippinensis* may in reality be one species. According to Weston, the main differences were that only *P. sacchari* infected sugarcane, and that oospores were known for *P. sacchari* but not for *P. philippinensis*. Crossing studies may later verify whether *P. sacchari* and *P. philippinensis* are the same species; however, to date we have observed the sexual stage only in the isolates of *P. sacchari*.

Isolate *P. sacchari* '83 differed from *P. sacchari* '75 and the two isolates of *P. philippinensis* for several enzymes. *P. sacchari* '75 differed from *P. sacchari* '83 at the Gpi, Mdh, Aat, Idh, Sod, and Pep-GL loci (Fig. 1, Table 2). The frequency of polymorphic loci detected for *P. sacchari* indicates that the species has considerable variation.

The Thai isolate of *P. sorghi* differed greatly from the five typical isolates of *P. sorghi* and had a maximum of only four alleles (coefficient of similarity [CS] = 0.24–0.33, Table 3) in common with any of the typical isolates of *P. sorghi* (Table 2). These include Gpt 100 (common to all isolates of *P. sorghi*), Gr 100 (common to *P. sorghi* In '80), and the alleles at the loci Pep-LLL-1 and Pgd. The latter two loci were monomorphic (no variation) to all isolates of *Peronosclerospora*. Indeed, the Thai isolate of *P. sorghi* appeared more closely related to *P. sacchari* and *P. philippinensis* where presumably eight alleles were in common (Table 2) and the CS averaged 0.50.

A list of presumed genotypes is presented in Table 2. Application of the technique of Rogers (18) to determine CS shows that all typical cultures of *P. sorghi* were truly closely related with 10 of 13 loci examined being monomorphic and CS values of 0.77 or higher (Table 3).

The major conclusions from this study are: that *P. sacchari* in Taiwan may be essentially the same pathogen as *P. philippinensis* in the Philippines; *P. sorghi* in Thailand genetically is very different

TABLE 3. Coefficients of similarity between genotypic classes of *Peronosclerospora* based on allelic protein variability at 13 loci[a]

Genotypic class	2	3	4	5	6	7
1	0.85	0.92	0.96	0.18	0.33	0.25
2		0.77	0.81	0.18	0.33	0.33
3			0.88	0.18	0.25	0.25
4				0.17	0.32	0.24
5					0.60	0.55
6						0.36

[a] Assumption is made that for each of 11 of the enzymes tested, a single locus codes for the enzyme. For PEP-LLL, two loci code for the enzyme with each isolate.

from *P. sorghi* in India, Brazil, and the United States and probably was misidentified; that the genus *Peronosclerospora* needs reevaluation in terms of delineation of species; and that with the acquisition and isozyme analysis of additional isolates of *Peronosclerospora*, the genetic relationships of pathogenic *Peronosclerospora* throughout the world can be ascertained. It is important to recognize that isozyme analysis is not a substitute for, but an adjunct to, morphological comparisons, and it is a powerful tool that presently is not being used adequately in the field of systematics of fungal plant pathogens.

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