Symposium

Population Genetics of Soilborne Fungal Plant Pathogens

The Population Genetics of *Phytophthora*

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There are more than 60 species in the genus *Phytophthora* (94), and most are destructive plant pathogens. Extensive efforts are directed at the control of Phytophthora diseases each year, yet they still cause serious crop losses. For example, more than $200 million in lost production annually is attributed to Phytophthora diseases in Australia alone (47). Late blight, caused by *P. infestans*, probably cost United States potato and tomato growers more than $200 million during 1994: $100 million in lost crop production and $100 million in additional control measures (30). Although usually remembered for the historical role played by *P. infestans* during the Irish potato famine of the 1840s, *Phytophthora* species still pose an immediate and real threat to world agriculture.

Despite the huge economic costs of Phytophthora diseases, relatively little is known about the population genetics of the causal organisms. Genetic analyses in this genus were hindered until recently by the inability to perform genetic crosses, due to homothallism, poor germination of oospores, lack of suitable markers, or, simply, the limited availability of both mating types (91). Biochemical markers were first applied to analyze populations of *P. cinnamomi* (77) and *P. infestans* (99) in the mid-1980s. Progress accelerated during the 1990s with the addition of DNA-based markers and the inclusion of more species. Although much remains to be learned, some general patterns are beginning to emerge.

The purpose of this paper is to review the literature on the population genetics of *Phytophthora* species and to formulate testable hypotheses that can explain many of the observed phenomena. Five main areas will be considered: sources of variation, migration, genetic drift, selection, and mating systems. Reviews of the genetics, systematics, and evolution of *Phytophthora* species have been published recently (9,10,48,50,91) and will not be duplicated here, except as needed to clarify particular points.

LIFE HISTORY OF *PHYTOPHTHORA*

Most *Phytophthora* species are ideally adapted to fulfill their roles as plant pathogens. They have a sexual cycle that results in the production of oospores. Their oospores are usually thick walled and resistant to environmental extremes. About half the species are homothallic and readily form oospores after colonization of host tissue. The rest are heterothallic and require two mating (compatibility) types to produce oospores. Oospores can survive at least for months (20,81) and probably for years in soil (24,25). Thus, they provide a long-lived source of inoculum (24) that can allow the otherwise near-obligate pathogens to survive outside their hosts.

In addition to sexual oospores, asexual propagules are produced in abundance on host tissue. Sporangia of some species are easily detached (caducous) and specifically adapted for aerial dispersal over wide distances. Sporangia of noncaducous species can be spread in water. Biflagellate swimming spores (zoospores) also are released from the sporangia under certain conditions (usually in response to chilling and free moisture). These spores are chemotactic and, thus, actively detect and swim toward suitable hosts (43). With oospores for surviving host-free periods, sporangia for long-distance dispersal, and zoospores that actively seek out hosts over short distances, it is not surprising that *Phytophthora* species are such formidable pathogens.

Most species of *Phytophthora* are soilborne root pathogens. A few infect aboveground plant parts but survive in soil as oospores. Additional details about the life history and biology of *Phytophthora* species can be found in several excellent reviews of the genus in general (9,48,89) and *P. infestans* in particular (4,33,91).

SOURCES OF VARIATION

Mutation

Mutation is the primary source of new genetic variation. Unfortunately, little is known about mutation or mutation rates in any Oomycete. However, clonal reproduction is predominant in populations of many *Phytophthora* species, and some information about mutation can be gleaned from analyses of genetic variation within clonal lineages.

All members of a clonal lineage are descended from a single individual; any variation within a lineage must arise by mutation (or possibly mitotic recombination; discussed below). So far, clonal lineages have been identified in *P. infestans* and *P. sojae*. The most commonly detected clonal lineage of *P. infestans* has been designated US-1 (35). It is an A1 mating type, has the dilocus allozyme genotype *Glucose-6-phosphate isomerase* (*Gpi*) *86*/*100*, *Peptidase* (*Pep*) *92*/*100* (92), and a characteristic 15-band DNA fingerprint (21,35,39). All members of the US-1 clonal lineage of *P. infestans* worldwide most likely were derived from a single Mexican ancestor during the past 154 years (36; discussed below). This places a probable upper limit on the time in which all changes within this lineage have occurred.

Analyses of US-1 isolates worldwide have detected a number of probable mutations both in nuclear and mitochondrial (mt) DNA. Variants within clonal lineages have been indicated by appending a period and a number after the lineage name (35). For example, US-1.3 is the third variant detected within the US-1 clonal lineage. These variants are identical to the most common genotype of their lineage, except for one or two allozyme or DNA finger-

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print changes (35,39). No mutations at allozyme loci have been detected within any lineage (although changes attributed to mitotic recombination have been observed; discussed below). However, three DNA fingerprint variants that probably arose by mutation have been identified. The US-1.5 (39) and US-1.6 (40) genotypes both contain extra DNA fingerprint bands compared to US-1: US-1.5 gained band 18, and US-1.6 gained band 22. The addition of a single fingerprint band also was found in a US-1 isolate collected in the United Kingdom in 1978 (21). A similar change was discovered within the US-6 clonal lineage: US-6.3 is identical to US-6, except for the gain of fingerprint band 12. Thus, at least four mutations at DNA fingerprint loci have occurred within clonal lineages of *P. infestans*.

Mutations also were detected in the mtDNA of *P. infestans*. Most US-1 isolates have mtDNA haplotype A (34) (called type Ib by Carter et al. [12]), and this is most likely the ancestral haplotype for this lineage. Analyses of total mtDNA after digestion with a single restriction enzyme revealed three mtDNA variants among US-1 isolates: the C form in a single isolate from Peru (34), and the E and F forms in isolates from the Philippines (54). The changes involve insertions and deletions; additional variants undoubtedly would be found in a systematic survey with more enzymes. Although these results are very limited, they do indicate that mutations in both nuclear and mtDNA occur at a high enough frequency to be detectable.

Even faster rates of genetic change due to mutation appear to occur for virulence. US-1 was probably the only genotype of *P. infestans* present in Europe prior to the late 1970s (discussed below), and the large amount of virulence variation identified in early European samples most likely resulted from mutation within the US-1 clonal lineage. For example, Malcolmson (64) identified 70 races of *P. infestans* among 373 samples collected in the United Kingdom from 1966 through 1968. Analyses of 200 isolates obtained primarily from North Wales during 1970 through 1973 identified 43 races (85). Additional virulence variation was identified among known US-1 isolates in the Netherlands (21), Peru (40, 102), Poland (97), and northern North America (40). Although the frequencies varied widely, pathogenicity to all 13 potato and tomato resistance genes tested has been identified within the US-1 clonal lineage (40). Similar rapid changes in virulence occurred within the US-7 and -8 clonal lineages in the United States and Canada (40).

Rapid rates of change due to mutation at virulence loci also have been discovered within *P. sojae* (23,28). Isolates of *P. sojae* from the United States that shared a single restriction fragment length polymorphism (RFLP) genotype were differentiated into seven races, six of which probably arose from race 1 by mutation (28). These isolates also had a number of mtDNA haplotypes (28), indicating mutations within the mtDNA also occurred rapidly within *P. sojae*. Among 84 Australian isolates tested, more than 95% share a common RFLP genotype and may be clonal descendants from a single introduction during the late 1970s (23) (these "clones" probably are propagated sexually; sexual and asexual progeny are indistinguishable in a highly inbred, self-fertilizing species such as *P. sojae*). Race 1 appeared to be the original phenotype; four additional races arose, probably by mutation and selection after migration of this clonal lineage to Australia.

Mutations also have occurred for fungicide resistance. Extreme sensitivity to metalaxyl seems to be the ancestral phenotype for the US-1 clonal lineage of *P. infestans*. However, metalaxyl-resistant US-1 isolates were obtained in the Philippines (54), where US-1 is the only known lineage. One of the first metalaxyl-resistant isolates collected in Ireland in 1980 (13) recently was shown to have the US-1 genotype (41). Mutations to metalaxyl resistance within the US-1 clonal lineage of *P. infestans* must be relatively rare, because they have not been detected yet in the United States (41).

Mutation rates do not need to be excessively high to explain the observed changes in virulence and fungicide sensitivity. A single lesion caused by *P. infestans* can produce hundreds of thousands of sporangia (58); an infected field could produce billions. With a huge number of propagules and strong selection imposed by host resistance genes and fungicides, a moderate mutation rate would be sufficient to generate all of the observed variation.

Mitotic Recombination

The changes described above involved the appearance of new alleles that must have been due to mutation. However, most of the variation within clonal lineages of *P. infestans* did not involve new alleles but, rather, apparent changes to homozygosity at loci that were heterozygous in the most common genotype (35,36). These changes could have been caused by mitotic recombination. A crossover during mitosis causes a change to homozygosity at all heterozygous loci on the same chromosome arm that are distal to the recombination break point. Although mitotic recombination does not generate new variation, it can reveal recessive variation that previously was hidden in heterozygotes.

Putative mitotic recombinants of *P. infestans* were detected more often than mutants (35,36). All of the observed allozyme variation within clonal lineages involved changes from heterozygosity to homozygosity; for example, from *92*/*100* to *92*/*92* at the *Pep* locus, as in the US-6.1 variant of the US-6 genotype (35). Additional changes were noted at DNA fingerprint loci that were heterozygous (+/–) and subsequently became homozygous (–/–), resulting in the loss of a band (35,36). Such changes also could be caused by mutation, e.g., by inactivation of an allozyme allele or by a change in restriction site at a DNA fingerprint locus. However, a very specific type of mutation would be required to effect these changes, which would probably occur much more rarely than mitotic recombination. Therefore, mitotic recombination is a much more likely explanation.

So far the mitotic recombination hypothesis has not been tested directly, primarily due to a lack of suitable markers. This hypothesis would be easy to test, because it makes specific predictions about the pattern of changes that should occur at linked loci. If mitotic recombination caused a change from *92*/*100* to *92*/*92* at the *Pep* locus, for example, then all heterozygous loci on the same chromosome arm distal to *Pep* also should become homozygous. In contrast, a mutation could occur without affecting nearby markers. Additional heterozygous markers linked to genes of interest will be needed to test the mitotic recombination hypothesis rigorously.

Parasexual Recombination

Another potential explanation for new variation within clonal lineages is parasexual recombination. There is some evidence of parasexual recombination in *Phytophthora* species in the laboratory (56,59,61,86), although it has not been confirmed with molecular markers. New races of another Oomycete, the lettuce downy mildew pathogen *Bremia lactucae*, appear to have arisen by somatic fusion (45), and somatic fusion followed by a parasexual cycle has been proposed to explain the recovery of new races of *P. infestans* in coinoculation studies (57,65). Shattock et al. (85) suggested that a high frequency of parasexual recombination could explain the apparent linkage equilibria observed among virulence phenotypes of *P. infestans* in Britain.

Although parasexual recombination is a possibility, it is not the most likely explanation for the majority of the observed changes. Each of the major events in the parasexual process (karyogamy in the heterokaryons, mitotic cross-over, and loss of extra chromosomes) occurs rarely, even in the model haploid organisms in which parasexual recombination first was identified (82). Therefore, it seems highly unlikely that parasexual recombination in *P. infestans* could occur at a high enough frequency to explain, for example, the results of Shattock et al. (85). Early reports of putative parasexual recombination for virulence markers also seem less likely when interpreted in the context of recent genetic data. Putative heterokaryons were identified by selecting for strains that could

grow on potato cultivars containing combinations of resistance genes that prevented infection by the original isolates (57,65); thus, the changes were always to increased virulence. This makes sense if virulence is dominant, as it may be at some loci (1,93). However, avirulence to potato resistance gene *R3* appears to be dominant in *P. infestans* (1,93); heterokaryons between isolates virulent and avirulent to *R3* should be avirulent, not virulent as has been reported (57,65). Therefore, mutation seems to be a much more plausible explanation for these results than parasexual recombination.

Fortunately, the parasexual recombination hypothesis can be tested easily with molecular markers. For example, previous studies (57, 65) could be repeated with isolates that differ for allozyme genotype. Allozyme analysis of any new races could determine whether they are heterokaryons. Dimeric enzymes, such as *Gpi* and *Pep*, are particularly useful for this purpose because the presence of heterodimer bands provides a sure method for distinguishing heterokaryons from physical mixtures of cultures (42). This is one of the advantages of allozymes over DNA-based markers. Clonal lineages of *P. infestans* appear to maintain their separate identities in the absence of sexual reproduction (21,35,36,38,39,54), so there is no evidence to support the high frequency of parasexual recombination that would be necessary to explain the results of previous studies.

Interspecific Hybridization

One final potential source of genetic variation is introgression by hybridization with other species. Interspecific hybrids between *P. infestans* and *P. mirabilis* have been made in the laboratory (37), and it has been hypothesized that *P. meadii* arose by interspecific hybridization (83), possibly between *P. botryosa* and another species. This is an interesting area of research that should be pursued more intensively in the future.

MIGRATION

Migration is a strong evolutionary force that has had a visible effect on the genetic structures of populations of *Phytophthora* species. Migration is the force that unites all members of a species in a common gene pool; relatively low levels of migration can prevent populations from diverging.

There are two components to migration: movement or dispersal of individuals and the contribution of migrants to the gene pool of the recipient population in subsequent generations. Gene flow only occurs if the migrant individuals become established and reproduce in the new population (26). Large migrations can result in little gene flow, and limited migration can have a large effect on gene flow, depending on the fate of the immigrant individuals after dispersal. The effect of migration also depends on the degree of differentiation among populations. Migrations that start new populations in previously unoccupied territories are called founder events. Founder populations typically contain only a small fraction of the genetic variation found in the original source populations. Because founder populations are usually small, genetic drift can occur during the founder event and in subsequent generations until the population size increases (66,73). Therefore, the genetic structures of founder populations are often very different from those in the original source populations.

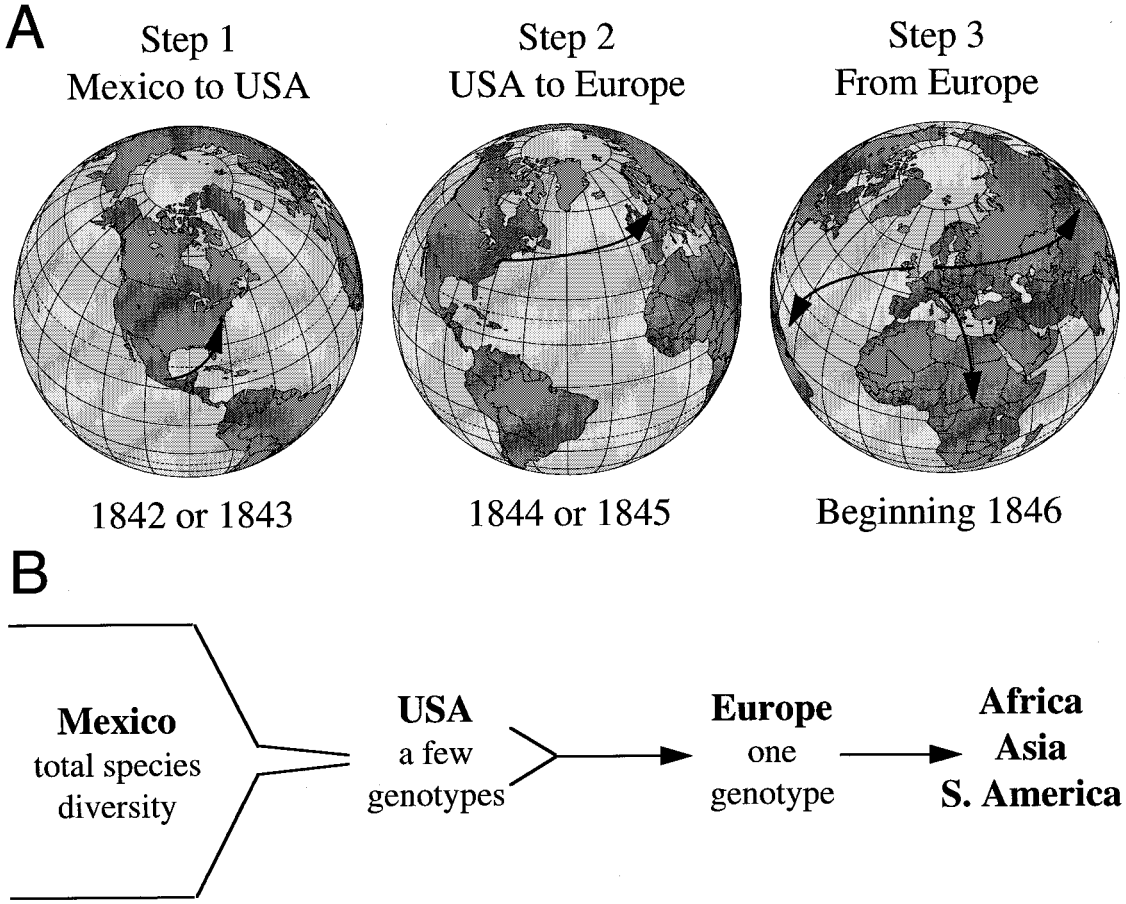

Fig. 1. Different stages in the initial panglobal migration of *Phytophthora infestans* and their effects on genetic diversity. **A,** The first migration probably occurred in three stages: (i) from Mexico into the northeastern United States in 1842 or 1843; (ii) from the United States into Europe in 1844 or 1845; and (iii) from Europe to the rest of the world after 1846. **B,** Severe genetic bottlenecks greatly reduced the level of genetic variation during the first two steps of the migration. Probably only a handful of genotypes (which did not include the A2 mating type) was introduced into the United States from Mexico and only one of these was transported subsequently from the United States into Europe. From Europe, a single clone probably was spread panglobally.

Mechanisms of Migration

Dispersal of *Phytophthora* species can occur due to many different mechanisms. Movement in infected plant parts is probably the most likely avenue for long-distance dispersal, particularly in woody or fleshy parts that do not dry out easily. For example, *P. infestans*, *P. erythroseptica*, and *P. parasitica* could be dispersed in potato tubers, *P. colocasiae* in taro corms, and *P. cinnamomi* in roots or adhering soil. Oospores are highly resistant to environmental extremes and could be dispersed in soil or dried plant parts. Homothallic species such as *P. phaseoli* and *P. sojae* may be particularly well adapted for dispersal in host materials; any host tissue could be a suitable substrate for oospore production.

Sporangia may be dispersed in water or aerially over long distances under favorable conditions, e.g., during thunderstorms when they would be relatively protected from desiccation and ultraviolet radiation. Zoospores have a much more limited range and probably are adapted for locating suitable host tissue over short distances, not as a means of dispersal.

Dispersal from Centers of Origin

Many *Phytophthora* species most likely evolved in limited geographic areas, and this is probably particularly true for those species that are host specific. Geographic ranges of most species have almost certainly expanded dramatically in historical times as the pathogens were dispersed in association with agricultural hosts. Thus, most migration events are probably very recent. Concepts of speciation and host-pathogen coevolution in *Phytophthora* have been discussed recently (10,48), so they will not be covered further here.

Because most *Phytophthora* species are near-obligate pathogens (hemibiotrophic), past migrations probably were limited both in occurrence and in the number of individuals introduced. Thus, all populations outside a species' center of origin most likely would be severely reduced in genetic variability and contain only a subset of the variation found in the primary center of diversity. Founder populations of heterothallic species would be limited to asexual reproduction if only one mating type was introduced; inability to produce oospores might make such populations more vulnerable to extinction. Homothallism, thus, may provide a selective advantage in colonizing populations. Recurring migration would diminish any differences among populations that might have developed due to founder events. Recurrent migrations at infrequent intervals can result in a gradual increase in genetic variation in the recipient populations or in a turnover of genotypes if new migrants are more fit than those already present. Examples of many of these phenomena can be found among *Phytophthora* species.

Global Migration of *P. infestans*

Most of the evidence of migration in *Phytophthora* species concerns *P. infestans* (30,32,33). This species evolved in a limited area in the highlands of central Mexico (75) but currently occurs throughout the world wherever potatoes and tomatoes are grown. Enough data have accumulated to propose hypotheses about the chronology and pathways of past migrations (30,32,33). Migrations out of Mexico probably occurred on at least four occasions beginning during the 1840s.

The first global migration of *P. infestans* most likely occurred in three stages (36) (Fig. 1A). Each stage was a founder event, because the fungus was being introduced into previously unoccupied territories. Migrations were noted by the appearance of late blight on potato crops, first in the eastern United States in 1843 (95), then in Europe in 1845 (8), and subsequently in almost all potato-growing areas worldwide (14). Because late blight is such a destructive disease, it seems highly unlikely that it could have occurred prior to the 1840s without being recorded. Thus, it is fairly certain that all long-distance migrations of *P. infestans* occurred during the past 154 years.

Historical and genetic data indicate that the first step in the initial migration probably was from Mexico into the United States (32, 36,95). Populations of *P. infestans* in the United States contain genotypes that appear to be related to the US-1 clonal lineage (and thus, are probably from the same source population) but that are not found anywhere else worldwide (35). Populations founded in this initial migration apparently experienced a severe genetic bottleneck (Fig. 1B) that greatly reduced their level of genetic variation.

Part of the lost genetic diversity probably included the A2 mating type. Oospores are a means of surviving host-free periods and, thus, provide a large fitness advantage for sexual compared to asexual populations. Because the maintenance of both mating types would have been favored strongly by selection, it seems most likely that only the A1 mating type was introduced.

There were two potential sources of *P. infestans* for the initial migration: (i) tubers of wild Mexican *Solanum* species and (ii) tubers of *S. andigenum* cultivars grown in small acreages in the high mountains of central Mexico since their introduction from the Andean region of South America during the 1790s (J. S. Niederhauser, *personal communication*). Another possible means of movement would have been in oospores, but it seems highly unlikely that oospore-contaminated soil would have been transported from Mexico into the United States. Transport of infected tubers could have been provided by one of the botanists or agronomists who visited Mexico during the nineteenth century and was interested enough in these "strange" potato plants to bring a few tubers home. Such importations would have been extremely limited, causing a severe genetic bottleneck for the fungus. This probably explains why it took more than 250 years after the introduction of potatoes into Europe before late blight appeared and also may explain the absence of the A2 mating type in the initial migrant populations.

The original source population for this migration is unknown, but it was most likely in central Mexico. Populations founded during the initial migrations are characterized by high frequencies of the allozyme alleles *Gpi 86* and *Pep 92* (36,92,99,102). These alleles occur at high frequencies in central Mexican populations of *P. infestans* (38,99) but not in populations at other locations in Mexico (38). Thus, the source population for the first migration of *P. infestans* probably was near the Toluca Valley in central Mexico.

The second step in the migration probably occurred during 1844 or 1845 with the introduction of a single clone from the United States into Europe (36). Because the source population for this migration probably was in the United States, the pool of genetic variation was extremely small. Historical evidence indicates that the initial outbreaks of late blight in the United States and Europe were in very limited geographic areas (8,95). This is consistent with genetic data indicating that the migrations were limited in size. Migration could have occurred in a single shipment of potatoes from one infected field. If other genotypes were introduced, they most likely died out quickly, because so far no evidence of other genotypes has been discovered. Potato cultivars grown in Europe during the 1840s were highly susceptible to late blight, and after the initial limited introduction, the fungus probably spread rapidly by airborne sporangia. If this hypothesis is correct, then the Irish potato famine was caused by a single clonal lineage of *P. infestans* (36).

After *P. infestans* had colonized Europe, it was transported to virtually every corner of the world in seed and ware potatoes (14). Because two successive genetic bottlenecks occurred in the founding of the European populations, almost no genetic variation remained when Europe served as the source population for the third wave of migration. The genotype transported during these early migrations was US-1 (35). US-1 isolates have been found in 18 countries on all the continents, except Australia and Antarctica (Table 1) (Taiwan is geographically separated from mainland China, so it was considered a separate country for this analysis). The US-1 genotype was the only one found in older collections of *P. infestans* dating back to the 1950s (Table 1).

The only exception reported so far is a single isolate collected in eastern Germany during 1977. This isolate was reported as having the *100*/*122* genotype at the *Gpi* locus (15). However, the *Gpi 122* allele has never been reported by other investigators outside Mexico, except for very recently in the United States (39). When tested at Cornell University, Ithaca, NY, this isolate was scored as *Gpi 86*/*100*, in accordance with the other isolates from that collection. Band shifting on an allozyme gel easily could cause one heterozygous genotype to be mistaken for another. Therefore, this report of the *Gpi 100*/*122* genotype in old German isolates may be an error.

Because opportunities for migration were so limited, there was probably no more gene flow of *P. infestans* out of Mexico until the 1970s. If other migrations occurred, they evidently did not become established. This may have changed during 1976 and 1977, when 25,000 tons of potatoes were shipped from Mexico to Europe (75). A second migration was first noticed in 1984, with the discovery of the A2 mating type in isolates from culture collections dating back to 1981 (44). Allozyme analysis revealed that other changes had occurred concomitant with the appearance of the A2 mating type (92). Isolates with the A2 mating type and new allozyme genotypes were collected in the Netherlands and eastern Germany as early as 1980 (15,21). These genotypes had spread throughout Europe (5,19,29,36,87,92,96,103) and to the Middle East (36), Africa (36), and South America (32) by the early 1990s. Evidence supporting this migration and the associated changes that occurred in *P. infestans* populations worldwide has been reviewed recently (21,22,31,32,33,84).

The genetic bottleneck for this second migration apparently was not as severe as the one during the 1840s. This is not surprising if the mechanism for migration was the 1976 and 1977 potato shipment. A large shipment of commercial potatoes is much more likely to contain more pathogen genotypes than a small shipment of wild potatoes. Furthermore, shipping times were shorter during the 1970s, which would increase the survival rate of the fungus. In addition to the A2 mating type, this migration introduced new A1 mating-type genotypes, the *Gpi 90* and *Pep 83* allozyme alleles (92), many more DNA fingerprint bands (19,36,96), and additional mtDNA haplotypes (19,22,34,84). Virulence diversity also increased (21,97), and previously resistant potato cultivars became susceptible (96,97). These new genotypes had greater fitness and rapidly replaced the old clonal lineage (32,84,92,96). Thus, this migration had a huge effect on gene flow.

A secondary result of this migration was the occurrence of sexual reproduction of *P. infestans* in Europe. Strong evidence of sexual reproduction has been found in Poland (96) and the Netherlands (21). All sexual recombination so far appears to have occurred exclusively among new genotypes, not between old and new genotypes (21,22,96). Evidently the new migrants contained both A1 and A2 mating types and did not need to recombine with the old A1 genotype. Recombination between the old and new populations may have been hindered by ploidy differences: the old population was predominantly triploid or tetraploid (101,102), whereas the new populations were predominantly diploid (15,97,103). A large mutational load in the old asexual population may have restricted recombination further (22). Gene flow in this case apparently occurred by replacement of the old populations by new migrants, with little or no introgression.

A third migration of *P. infestans* out of Mexico appears to have occurred during the late 1970s, this time from northwestern Mexico into the United States (35). Evidence for this migration is less certain than for the previous two. Late blight epidemics occurred on southern California potato and tomato crops in 1979 after a 32-year absence (105,106). One isolate from California in 1982 had the US-6 genotype, which had not been detected previously in the United States (35). However, US-6 was by far the most common genotype in northwestern Mexico in 1989 (38). All US-6 isolates tested so far infect both potato and tomato, whereas most US-1 isolates only infect potato (40). The appearance of new late blight epidemics on potato and tomato after a 32-year absence, coupled with the recovery of the previously unknown US-6 genotype, which is highly pathogenic to both crops, provides strong circumstantial evidence that these epidemics were caused by the migration of new genotypes, probably from northwestern Mexico. However, samples prior to 1980 are very small, and this conclusion needs to be verified by additional analyses.

In contrast, evidence for the fourth migration is quite clear. This migration brought new genotypes from northwestern Mexico into the United States and Canada. Two new genotypes, US-7 and -8, were detected in the United States beginning in 1992 (39). These genotypes were of the A2 mating type and were highly resistant to the fungicide metalaxyl (41). (A2 mating-type isolates were first detected in the United States and Canada during 1987 and 1989, respectively [17], but the genotypes of these isolates were not determined.) Both genotypes had spread throughout the United States and into Canada by 1994 (31,37). Movement of these genotypes into the United States initially was probably in infected tomato fruits. Tomatoes are imported into the United States from northwestern Mexico, and late blight-infected fruits from Guasave, Sinaloa (in northwestern Mexico), were intercepted in Charleston County, SC, during April 1995 (A. P. Keinath, *personal communication*). Once in the United States, movement may have continued on tomato fruits or on transplants. The US-8 genotype is a very efficient colonizer of potato tubers, and the movement of this genotype in infected seed tubers probably contributed to its rapid increase from 1993 to 1994 (31,39). Both genotypes were spread rapidly by airborne sporangia after their initial introductions.

TABLE 1. Numbers of US-1 isolates of *Phytophthora infestans* confirmed with molecular markers in 19 countries on 4 continents worldwide

Location	Year(s)	Confirmed with allozymes	Confirmed with DNA fingerprints	Reference(s)[a]
Africa				
Rwanda	1984–1987	17	7	36
Asia				
China	Before 1982	6	6	54
Japan	1958–1983	4	…[b]	69
	1987	13	…	69
	1988	15	15	36, 54, 69, 92, 98
	1989	28	…	69
	1990	14	…	69
Korea	1991	1	1	54
Philippines	1989, 1991	28	28	36, 52
Taiwan	1991	3	3	54
Europe				
France	1990	1	1	5
Germany	1976–1980	10[c]	10	15, 21
Ireland	Before 1984	2	1	36, 99
Netherlands	1951–1978	7	5	21, 92
	1980–1982	2	2	36, 92
Poland	1985–1988	35	30	36, 92, 96
Russia	1979, 1990	3	3	36
Switzerland	1977, 1981	3	2	36
United Kingdom	Before 1980	…	6	21
	Before 1984	8	5	36, 99
North America				
Canada	1980, 1982	3	3	36, 99
United States	1946–1989	72	17	35, 36, 92, 99
	1992–1993	115	48	39
South America				
Brazil	1986, 1987	4	3	36
Ecuador	1990–1992	5	3	W. E. Fry
	1993–1994	1	…	W. E. Fry
Peru	1982, 1984–1986	45	26	36, 102

[a] Sometimes the same isolates were used in different studies. All studies that used the same isolates are cited together.
[b] Not tested.
[c] One isolate was scored as *Glucose-6-phosphate isomerase* (*Gpi*) *100/122* by Daggett et al. (15). However, this isolate had the same DNA fingerprint as US-1 (21) and was most likely *Gpi 86/100* (discussed in text).

As in Europe, there is now evidence that sexual reproduction may be occurring in the United States and Canada. Most fields sampled contained only one mating type (16,35,39), so opportunities for sexual reproduction probably were extremely limited. However, analysis of a limited sample of isolates from British Columbia, Canada, identified four genotypes that may have arisen by sexual recombination (39). Additional genotypes that could have been generated by sexual recombination were identified in New York in 1994 (S. B. Goodwin and W. E. Fry, *unpublished data*), although it is difficult to determine whether sexual reproduction occurred in situ or recombinant genotypes were imported from elsewhere. Sexually reproducing populations of *P. infestans* may become established in many parts of the United States and Canada in the near future.

Recent studies provide tantalizing evidence for other possible migrations of *P. infestans* from Mexico. Populations in Asia are composed of two different clones, US-1 (the clone that occurs throughout the world) and JP-1 (54). The A2 mating-type JP-1 clone is now dominant in Korea and Japan (54). Interestingly, although both mating types have been present in Japan at least since 1987 (68) and the clones containing them are sexually compatible in the laboratory (69), so far there is no evidence of sexual recombination in the field (54,69). The JP-1 clone in Asia is quite different from genotypes in other areas (36) and may have originated from a separate introduction. Similarly, isolates representing single clones in Australia, Bolivia, Brazil, and Costa Rica have unusual genotypes (38) that probably were not introduced during any of the four known migrations. However, the genetic diversity in these populations is still a subset of that in Mexico.

Migrations of Other *Phytophthora* Species

Evidence of past migrations has begun to accumulate for other *Phytophthora* species. Old et al. (77) found that populations of *P. cinnamomi* in Australia contained only four multilocus isozyme genotypes, whereas nine genotypes and many more alleles were found in a limited sample from Papua New Guinea. The four genotypes in Australia may be separately evolving clones; no evidence of sexual reproduction was found in a large sample from different areas of the country (77). If Papua New Guinea is the center of origin of *P. cinnamomi*, then the Australian populations most likely arose from a limited number of introductions. This study was the first to use molecular markers for analysis of *Phytophthora* population biology and also provided the first unambiguous genetic evidence of diploidy.

The lack of diversity for isozyme markers in Australian populations of *P. cinnamomi* was confirmed by a later study with an even larger sample size (76). Only three multilocus genotypes were identified, which were identical to three of the four Australian genotypes found in the first study. This confirmed that introductions of *P. cinnamomi* into Australia were probably extremely limited. Even though both mating types were widely distributed in Australia, opportunities for sexual reproduction may have been minimal, because both mating types were found together only at one location in New South Wales (76). Even there, there was no evidence of sexual reproduction (76).

Recent analyses of low-copy RFLP markers in *P. sojae* have revealed much less diversity in Australia than in the United States (23). In fact, most of the diversity in Australia occurred within a single clonal lineage, similar to the variation in *P. infestans* after the global migration of the US-1 clonal lineage. On the basis of these results, Drenth et al. (23) hypothesized that *P. sojae* in Australia may have originated from a single introduction.

Migration of *P. cambivora* may have gone in the other direction. There was more diversity among isolates of *P. cambivora* from Australia compared to those from other locations (78); all isolates from Europe had a single multilocus isozyme genotype. Thus, European populations of *P. cambivora* may have originated from a single introduction from Australia (78) or another location. Restricted migration from geographically limited centers of origin seems to be a common theme for many *Phytophthora* species, although the number of species studied is limited.

GENETIC DRIFT

There are two main causes of genetic drift in *Phytophthora* populations: (i) seasonal reductions in population size due to unfavorable environmental conditions and (ii) founder effects. Founder effects have been discussed under migration, so they will not be discussed further. Fluctuations in population size probably would be most noticeable for *Phytophthora* species that infect herbaceous hosts. Such hosts survive cold and dry seasons in perennial tissues (roots, corms, and tubers) or as seeds. When host tissue becomes limited, pathogen populations crash, persisting in infected plant parts in soil or as oospores. Genetic drift results if the next population is started from a small number of surviving individuals. Because oospores increase the probability of survival, genetic drift would be most severe for heterothallic species when only one mating type is present.

There is some evidence that drift affects the genetic structure of populations of *P. infestans*. The same genotypes were rarely detected in the same place from one year to the next in the United States and the Netherlands (21,39), indicating the probable action of genetic drift. However, it is difficult to separate the effects of genetic drift from those of other forces. Much more study is needed before the full effects of genetic drift on populations of any *Phytophthora* species can be estimated.

SELECTION

The fate of genes introduced into a population through mutation or migration is determined in part by selection. Variants with higher fitness increase in frequency; less fit variants decrease in frequency and eventually are eliminated. Selection can cause rapid changes in populations when fitness differences are large.

One of the obvious causes of selection in *Phytophthora* species is host resistance genes, which has been particularly well documented for *P. infestans*. The only virulence genes expressed by US-1 isolates of *P. infestans* in the Netherlands were those that could overcome resistance genes present in commonly grown potato cultivars (21). Similarly, the rapid increase in virulence to resistance genes *R1* to *R3* in Great Britain closely followed the introduction of those genes into potato cvs. Pentland Dell (*R1* to *R3*) and Maris Peer (*R1* and *R2*) (85). Tooley et al. (99) believed that the high frequency of virulence to resistance gene *R1* in Peru was due to the prevalence of this gene in Peruvian potato cultivars. Thus, there is ample evidence that *R*-gene selection has occurred in *P. infestans* (32), and it probably also occurs in other *Phytophthora* species.

In Australia, the occurrence of identical races of *P. sojae* in different genetic backgrounds was interpreted as evidence of convergent evolution (23). This term usually is applied when similar traits have evolved in different species. However, in the absence of recombination, the various clones of *P. sojae* are independently evolving units, so use of the term convergent evolution may be appropriate. In that case, there is strong evidence that convergent evolution for race phenotype has occurred within *P. sojae* in Australia (23,48). This phenomenon probably would occur wherever cultivars with similar resistance genes are used.

Mechanisms of Population Replacements

Strong selection that can result in rapid turnover of genotypes within populations has been observed in *P. infestans*. The US-1 clonal lineage of *P. infestans* does not compete well against introduced variants and, in fact, has been replaced rapidly by new genotypes in Europe (21,84,92,96), Asia (54), South America (33), and, more recently, in the United States (35,39). The time from the first detection of new migrants to the complete replacement of US-1 was only a few years (32). Because the new migrant geno-

types rapidly replaced the US-1 clonal lineage, they must have had a large fitness advantage (32,92). However, the exact mechanism for this fitness advantage has not been determined.

At least three mechanisms seem likely. The first is sexual reproduction. Most *Phytophthora* species are near-obligate pathogens. Their ability to produce oospores eliminates dependence on host tissue and may greatly increase both the amount and longevity of inoculum compared to clonal reproduction. This advantage in itself could account for the rapid replacement of asexual by sexual populations regardless of the benefits that may accrue due to recombination (21). Because there were no concomitant changes in host genotypes at the time of these migrations (21), other types of selection (such as *R*-gene selection) are very unlikely (32,92).

The second potential mechanism is Muller's ratchet (22,27,71). Because most mutations are deleterious, they decrease the average fitness of individuals in a population (108), resulting in mutational load (70). Sexual recombination can produce individuals with fewer deleterious mutations and, thus, higher fitness (71). However, in asexual populations, progeny would always have at least as many deleterious mutations as their parents (71). Because asexual populations have no mechanisms for removing deleterious mutations, they would accumulate over time. Thus, asexual populations would be composed of the original clone plus other clones with lower fitness due to mutation. If the most fit genotype is lost by chance (genetic drift), then the fitness of the population as a whole would decrease; Muller's ratchet would have clicked one step (63). Under certain conditions the mutation rate can be on the order of one per generation—high enough to drive the ratchet by itself (62). Therefore, the most fit genotype could be lost due to mutation pressure alone, in addition to genetic drift (62).

The ratchet clicks faster with small effective population sizes (63), because small populations are more sensitive to genetic drift and mutation pressure (62). This can set up a kind of positive feedback mechanism: as population fitness decreases, population size also decreases, increasing the speed of the ratchet (63). The eventual result may be "mutational meltdown" (63) and extinction of the population (Fig. 2). Selection against unfavorable mutations may slow the ratchet and allow asexual populations to survive indefinitely but with a lower mean fitness than in sexual populations (55).

Some asexual populations of *P. infestans* could be near mutational meltdown. If there are 10 cycles of asexual reproduction per year, then populations containing the US-1 genotype may have undergone more than 1,500 generations of asexual reproduction during the 150 years since the probable migration of this genotype from Mexico. Mutational meltdown can occur after 10^4 to 10^5 generations of asexual reproduction (63) and in even fewer generations under certain conditions (62). Thus, US-1–dominated populations of *P. infestans* probably have a large mutational load (22), which could explain their apparently low fitness compared to recent migrants from sexually reproducing populations in Mexico.

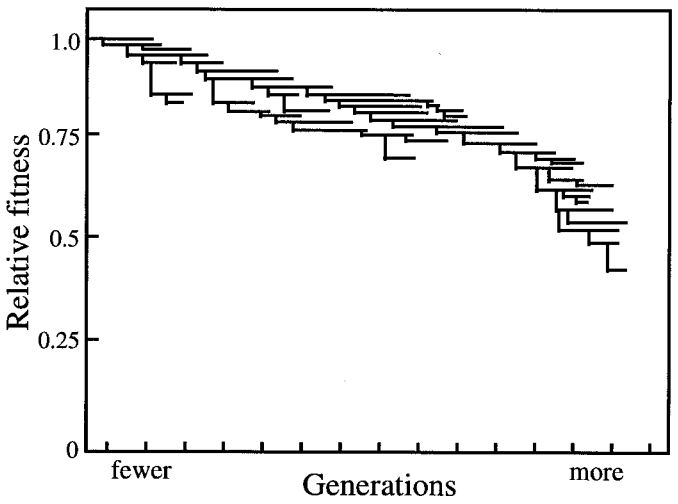

Fig. 2. The effect of Muller's ratchet on population fitness. Muller's ratchet gradually reduces the mean fitness of clones in an asexual population. New clones with lower fitness are generated constantly by mutation. The ratchet clicks when the most fit genotype is lost by chance (genetic drift) or mutation pressure. Eventually, the mean fitness becomes too low for the population to be self-sustaining. As population size decreases, the ratchet clicks faster, leading to "mutational meltdown" and extinction (62). This drawing illustrates the concept only and is not based on real data.

A third possibility is that genetic bottlenecks alone caused the reduction in fitness. Populations of *Drosophila pseudoobscura* that experienced a bottleneck had lower competitive fitness against populations of *D. willistoni* than those that had not experienced a bottleneck (60). The magnitude of the fitness reduction was related to the size of the bottleneck; populations founded by the minimum population size (i.e., one pair) had the lowest fitness (60). The two migrations of *P. infestans* into Europe also appear to have had bottlenecks of different sizes: the first migration probably had the absolute minimum population size of one genetic individual, whereas the bottleneck for the second migration was not as severe (although the amount of variation introduced during the second migration was still only a small subset of that in Mexico). If genetic bottlenecks alone cause a reduction in fitness, then populations founded by later migrations of *P. infestans* that did not appear to have experienced such a severe bottleneck may have had higher fitness compared to earlier populations that were probably founded by a single clone.

Fitness Components

The studies discussed above were all concerned with total fitness. However, there may be components of fitness at each stage of the life cycle that contribute to overall fitness. Fitness components have not been well studied in *Phytophthora*, and the few studies done with *P. infestans* can be difficult to interpret. For example, no differences were detected for overall fitness among isolates of *P. infestans* from asexual populations (mostly with the US-1 genotype) compared to those from sexual populations in central Mexico (100), even though there were differences in individual components. Differences in some fitness components were detected between old and new genotypes in the United States (53). US-1 isolates had longer latent periods compared to those with the US-7 and -8 genotypes and also produced smaller lesions with less sporulation than US-8 isolates (53). Furthermore, metalaxyl-resistant isolates produced significantly larger lesions than did sensitive isolates in Israel (52), although they may not survive as well as sensitive isolates in potato tubers (51). Genotypes that are highly fertile and produce more oospores may have a fitness advantage where both mating types occur.

Another component of fitness that may be important is host range. The ability of *P. infestans* genotypes to infect tomato in addition to potato may increase the area of host tissue available for colonization, which could be an advantage where both crops are grown. Isolates of *P. infestans* have been obtained from hairy nightshade (*S. sarrachoides*), a common weed in many parts of the United States (18,39,105); differences in ability to infect this or other alternative hosts could provide large fitness differences in the field. All aspects of the life cycle must be evaluated for the effect they have on overall fitness. This is an important area of research that will provide essential information for understanding the mechanisms of population replacements.

MATING SYSTEM

Phytophthora species are either heterothallic or homothallic. Heterothallic species require the interaction of two mating (compatibility) types to initiate sexual reproduction and, thus, are expected to reproduce predominantly by outcrossing. In contrast, homothallic species can initiate sexual reproduction singly by self-fertiliza-

tion and, thus, should be highly inbred. Variations on this simple theme also occur. Homothallic isolates have been identified in many heterothallic species (9), and self-fertilization in heterothallic species can occur even in pairings between isolates of opposite mating types (37,88,90). In addition, recent analyses indicate that homothallic species can outcross (7,104,109,110). Thus, the amount of inbreeding that occurs within populations of *Phytophthora* species in nature may vary and cannot necessarily be predicted on the basis of hetero- and homothallism.

Self-fertilization reduces the amount of heterozygosity by one-half in every generation. In only seven generations of self-fertilization, less than 1% of the original heterozygosity should remain. This predicts that there should be almost no heterozygosity in populations of homothallic *Phytophthora* species that have been established for more than a few generations. Once near homozygosity, there is little difference between sexual and asexual reproduction (other than spore type), so populations would remain clonal regardless of which type of reproduction predominates.

Heterothallic species, on the other hand, should contain high levels of heterozygosity. A single generation of random mating will return the level of heterozygosity to that expected under the Hardy-Weinberg equilibrium. Species in which mixed outcrossing and selfing occurs should contain intermediate levels of heterozygosity, depending on the amount of outcrossing that actually occurs. However, levels of heterozygosity within *Phytophthora* species have not been studied.

Fixation Index

One way to analyze the mating system is by comparing the observed proportion of heterozygotes in a population to that expected assuming random mating. Wright's fixation index (F) is a convenient estimate of genotypic structure, which is calculated as $F = 1 - (H_{Obs}/H_{Exp})$, where H_{Obs} is the observed mean heterozygosity per locus and H_{Exp} is the expected proportion of heterozygotes assuming random mating (11). H_{Exp} also has been called "virtual" heterozygosity and is the same as Nei's gene diversity (72).

Under complete selfing, the observed level of heterozygosity should be near 0, and F should be near 1. Under random mating, H_{Obs} should be close to H_{Exp}, and F should be near 0. Values of F between 0 and 1 indicate various levels of inbreeding. A value of F less than 0 indicates an excess of heterozygotes, either due to disassortative mating or the predominance of a particularly fit heterozygous clone. Asexual reproduction could skew F values in any direction depending on the level of heterozygosity present in the clones with the highest fitness. In addition to selfing, matings between close relatives also would increase the values of F.

To test whether there are differences in mating system among *Phytophthora* species, Wright's fixation indices were calculated for 16 species by reanalysis of previously published data (Table 2). As expected, fixation indices were near 1.0 for four of the six homothallic species studied (Table 2). The exceptions were *P. heveae* and *P. cactorum*. Only two loci were heterozygous in *P. heveae*, and most of the heterozygosity was at the *Malate dehydrogenase-1* (*Mdh-1*) locus (79). It is possible that *Mdh-1* heterozygotes have a fitness advantage and were maintained at a high frequency by selection, even though most other loci became homozygous. With a fixation index of 0.69, *P. heveae* probably has a mixed mating system (inbreeding and outcrossing) with a predominance of self-fertilization. However, the sample size was extremely limited (n = 14), and this hypothesis needs to be tested further by analysis of additional samples.

The other exception was *P. cactorum*, with a fixation index of –0.51 (Table 2). All of the heterozygosity in *P. cactorum* was at the *Pep* locus (78); in fact, every isolate was scored as a *Pep* heterozygote. Unless reproduction is exclusively asexual, it seems highly unlikely that selection could maintain fixed heterozygosity at a single locus, whereas all other loci become homozygous. A more likely explanation is that the banding pattern at the *Pep* locus was due to multiple loci rather than heterozygosity. This could be tested by examining single-oospore progeny for segregation. If no segregation occurs, then this locus is not heterozygous and the fixation index becomes 1.0, as expected under complete self-fertilization.

Fixation indices for 4 of the 10 heterothallic species were between 0 and 0.3 (Table 2), as expected for random mating populations (11). This includes samples of *P. infestans* and *P. cinnamomi* from their putative centers of origin. The remaining six species could be divided into three groups: those with intermediate fixation index values near 0.5; those with high fixation index values similar to homothallic species; and *P. palmivora* with negative fixation indices (Table 2). The first group contains *P. citrophthora*, *P. meadii*, and *P. megakarya*. The most likely explanation for the intermediate fixation index values of these three species is that they have a mixed mating system that includes a moderate amount of inbreeding in addition to outcrossing.

The high fixation indices for *P. botryosa*, *P. capsici*, and one Australian sample of *P. cinnamomi* (Table 2) indicate a significant deviation from random mating. For *P. cinnamomi* in Australia, the most likely explanation is asexual reproduction dominated by clones with low levels of heterozygosity rather than selfing. However, for *P. botryosa* and *P. capsici*, the most likely interpretation is that a high level of inbreeding occurs in natural populations. This does not necessarily imply self-fertilization. Outcrossing among genetically related individuals can result in a high fixation index without selfing. This could occur, for example, if a population has experienced a genetic bottleneck. Soil-inhabiting *Phytophthora* species such as *P. capsici* may be particularly prone to inbreeding, because dispersal may be more limited.

A negative fixation index was obtained for *P. infestans* outside Mexico and for *P. palmivora* (Table 2). For *P. infestans*, this is almost certainly the result of asexual propagation of a heterozygous clone, resulting in a large excess of heterozygosity. For worldwide isolates of *P. palmivora*, the 106 isolates tested were

TABLE 2. Fixation indices calculated from previously published isozyme and restriction fragment length polymorphism (RFLP) data for 16 species of *Phytophthora*

Species (reference)	Sample size	No. of loci	Heterozygosity		Fixation index
			Actual	Expected	
Homothallic species					
P. boehmeriae (79)	11	12	0.015	0.32	0.95
P. cactorum (78)	47	18	0.056	0.037	–0.51
P. citricola (80)	125	14	0.007	0.279	0.97
P. heveae (79)	14	17	0.05	0.16	0.69
P. katsurae (79)	16	17	0.00	0.11	1.0
P. sojae (28)	48	15	0.00	…[a]	1.0
Heterothallic species					
P. botryosa (79)	10	18	0.006	0.08	0.93
P. cambivora (78)	25	18	0.060	0.071	0.15
P. capsici (79)	84	18	0.019	0.20	0.91
P. cinnamomi					
Worldwide (78)	81	18	0.098	0.134	0.27
Australia (77)	165	20	0.010	0.049	0.80
Australia (76)	280	19	0.051	0.115	0.56
PNG[b] (77)	18	20	0.058	0.088	0.34
P. citrophthora (79)	43	18	0.107	0.20	0.47
P. infestans					
Mexico (99)	50	15	0.037	0.046	0.20
Non-Mexican (99)	46	15	0.120	0.066	–0.82
P. meadii (79)	33	18	0.066	0.12	0.45
P. megakarya (79)	15	17	0.078	0.19	0.59
P. palmivora					
Worldwide (79)	106[c]	17	0.111	0.08	–0.39
East Asia (67)	62[d]	17	0.135	0.122	–0.11
P. parasitica (79)	60	19	0.079	0.11	0.28

[a] Förster et al. (28) did not provide genotype or allele frequencies, so it was impossible to calculate the expected heterozygosity for the RFLP data.
[b] Papua New Guinea.
[c] Includes six isolates of *P. arecae*, as described in Oudemans and Coffey (79).
[d] McHau and Coffey (67) reported data for 99 isolates, but 34 of these were published previously by Oudemans and Coffey (79), including the 6 isolates of *P. arecae*. This analysis includes only the 62 isolates from East Asia (67) that were not published previously.

scored as heterozygous at the *Pep* locus (79). However, because genetic analyses were not performed, it is possible that this was actually due to more than one *Pep* locus rather than to heterozygosity, as discussed for *P. cactorum*. If the heterozygosity at the *Pep* locus is removed, the fixation index for *P. palmivora* becomes 0.35, in accordance with random mating. Samples of *P. palmivora* from East Asia showed possible random mating, with a fixation index of –0.11 (Table 2), which may support the hypothesis (67) that this area is the center of origin for this species.

One other possible explanation for some of the results must be considered: the Wahlund effect. Samples of most species analyzed to date have been limited and designed to address questions about taxonomy rather than population genetics. Inadvertently combining samples from subpopulations that have different allele frequencies causes the Wahlund effect: a deficiency of heterozygotes (107) and, thus, a higher fixation index. This most likely affected the results for *P. citrophthora* and *P. megakarya*, in which different subgroups were identified (79). Although subgroups also were identified within *P. capsici* (79), there appeared to be a deficiency of heterozygosity within subgroups, consistent with a high level of inbreeding. Overall, these data indicate that many *Phytophthora* species in nature probably have a mating system that includes both inbreeding and outcrossing. However, this hypothesis needs to be tested by analysis of larger samples from carefully defined populations.

Excess Heterozygosity in Homothallic Species

There is one apparent contradiction that comes from this analysis. Bhat et al. (6) reported segregation for virulence in selfed progenies from two randomly chosen field isolates of *P. sojae*. Unfortunately, their results were not confirmed with unambiguous genetic markers and, so far, have not been supported by other investigators (110). If it can be confirmed, the most likely explanation for segregation in selfed progenies is that the original isolates were heterozygous (6). However, based on RFLP markers, the fixation index for *P. sojae* was 1.0 (Table 2). No heterozygotes were detected among 48 isolates at 32 putative genetic loci: 15 loci with known codominant alleles, 6 loci with inferred codominant alleles, and 11 loci with dominant alleles (28). This apparently high level of heterozygosity for virulence loci in a species that should be completely homozygous has not been explained.

One potential explanation is that there is selection for heterozygotes ("overdominance") at virulence loci (or at other loci closely linked to them). Overdominance at small chromosomal segments containing virulence loci could maintain higher than expected heterozygosity at those loci, even as the rest of the genome becomes homozygous through self-fertilization. This phenomenon (3) has been demonstrated in self-fertilizing populations of diverse plant species, including lima beans (2), barley (49), and wild oats (46), which have fixation index values (11) similar to those for homothallic *Phytophthora* species (Table 2). Although this hypothesis provides an explanation for the observed low level of heterozygosity for molecular markers (28,110) and the apparently high heterozygosity for virulence genes (6), it needs to be tested by more rigorous experimentation.

CONCLUSIONS

Modern analyses of the population genetics of *Phytophthora* species began during the mid-1980s when the first papers on isozyme variation in *P. cinnamomi* (77) and *P. infestans* (99) were published. Much has been learned since then, particularly during the last few years with the application of DNA-based markers.

Despite all that has been discovered, much remains to be learned; the study of *Phytophthora* population genetics is still in its infancy. Many unanswered questions have been indicated above. The following is a list of nine specific questions or areas of study that should be investigated in the future.

(i) Where is the center of origin and/or diversity for *Phytophthora* species? Although it is now quite certain that *P. infestans* evolved in central Mexico, the origin for most other species remains obscure. Both *P. cinnamomi* and *P. sojae* probably were introduced into Australia, possibly from Papua New Guinea and the United States, respectively, but it is not known whether these were primary or secondary centers of diversity. European populations of *P. cambivora* appear to be clonal and could have been derived from those in Australia. However, sampling from the probable centers of diversity and alternative locations for virtually all species has been extremely limited. Hypotheses about the putative centers of origin can be proposed on the basis of host ranges, mating-type distribution, and the limited genetic data available (10,111). These hypotheses should be tested by more extensive sampling for other *Phytophthora* species.

Knowing the center of origin for a pathogen is of more than academic interest. The center of origin most likely also would be the area of highest genetic diversity. Knowledge of the range of genetic variability in a center of diversity might indicate the potential for damage due to additional migrations and would indicate which regions should be monitored to minimize migrations. Centers of diversity are also the best places to look for new sources of resistance and to screen potentially resistant germ plasm. Knowing the ecology of the pathogen in its center of origin might help identify potential new strategies for disease management, such as other organisms that could be used for biological control.

(ii) Several specific questions about the migration of *P. infestans* remain. For example, the data clearly indicate that a second migration occurred from Mexico into Europe during the 1970s, probably when 25,000 tons of potatoes were exported from Sinaloa (in northwestern Mexico) during 1976 and 1977 (75). However, the exact Mexican source population for that migration has not been identified. It was probably not the Toluca Valley region of central Mexico, because allozyme alleles characteristic of the migrating populations (*Gpi 90*, *Pep 83*) do not occur at a high frequency in Toluca (38,99). Furthermore, the B form of mtDNA, which is common in northeastern and northwestern Mexico (34) and which was introduced during this migration (21,34,84), also has not been found in central Mexico (34). Sinaloa probably was not the source population for the migrant genotypes, because neither of the allozyme alleles characteristic of the migrating populations has been found there (38). Furthermore, it seems unlikely that the A2 mating type was present in Sinaloa in 1976, because only A1 isolates were identified in surveys made between 1950 and 1970 (75).

It is much more likely that other potato-producing regions of Mexico were the source population for the migrant genotypes. The commercial potato acreage in Sinaloa was expanding during the 1970s, and most fields were planted with seed tubers brought in from other regions of Mexico, including Toluca, Guanajuato, and Zamora, where the A2 mating type has been reported since the 1950s (74). Furthermore, Sinaloa was not a huge potato-producing region in 1976, and there is a good possibility that to complete the shipment of 25,000 tons from Sinaloa, it was necessary to bring in potatoes that were actually produced in the other regions during that year (J. S. Niederhauser, *personal communication*). However, isolates from Guanajuato and Zamora have not been analyzed with molecular markers. If one of these regions was the source for the 1970s migration, sampling should reveal a high frequency of the allozyme alleles *Gpi 90* and *Pep 83*, the B form of mtDNA, and a large diversity in DNA fingerprint types. Of course, the original source population for the 1970s migration may no longer exist, but if it does, its discovery would solve one of the remaining mysteries of the 1970s migration.

(iii) The Australian *P. infestans* population is another population about which very little is known. Isolates from Australia had an allozyme genotype very similar to the old US-1 clonal lineage but a very different DNA fingerprint (36). Australia is the only con-

tinent (other than Antarctica) from which the US-1 genotype has not been recovered. One possibility is that this genotype was never introduced into Australia and that Australian populations of *P. infestans* arose from different migrations than those in other parts of the world. However, sampling from Australia has been extremely limited, and there is not enough information available even to formulate a likely hypothesis.

(iv) There are still many unanswered questions about the sources of variation within populations of *Phytophthora* species. Very little is known about mutation or mutation rates in any *Phytophthora* species, and although mitotic recombination seems a likely hypothesis, the role actually played by mitotic recombination or parasexuality is not clear. Whether mitotic recombination is an important source of "new" variation within clonal lineages of *Phytophthora* species is an interesting hypothesis that could be tested easily with suitable markers, particularly once genetic maps are available.

(v) Selection obviously plays an important role in the population genetics of *Phytophthora* species but has not been studied extensively. Analysis of fitness components is a highly promising area of research, because it could help answer questions about the population replacements that are probably a common aspect of *Phytophthora* biology. The most important fitness components are likely to be latent period, lesion size, sporulation capacity, efficiency of tuber infection (for *P. infestans*), overwintering survival, sexual fertility, and host range.

The answers to these questions also have immediate practical significance. For example, programs for predicting late blight epidemics (e.g., BLITECAST, The Pennsylvania State University, University Park, PA) are an integral part of disease management strategies and can be used to dictate fungicide spray schedules. However, these management strategies were all designed and verified against previous populations of *P. infestans*, which we now know contained a single clone in many parts of the world. The new genotypes may have different temperature optima, shorter generation times, or better tuber-colonizing abilities that give them a fitness advantage. Expert systems need to take into account recent changes in the biology of the pathogens that may have been the result of these migrations. Knowing which components of fitness are different in new migrant genotypes may be important for improving disease management strategies.

(vi) The Muller's ratchet hypothesis for reduced fitness of established clonal lineages needs to be tested rigorously. If the fitness of clonal populations does decrease over time, then extra care to prevent new introductions may be warranted, even where a pathogen is already present. Anything that decreases the size of pathogen populations could increase the rate at which the ratchet clicks and, thus, help to lower pathogen fitness.

(vii) One of the important unanswered questions about the mating biology of heterothallic *Phytophthora* species is why both mating types can apparently coexist in some areas without sexual recombination. This has been observed in many populations of *P. infestans* and in Australian populations of *P. cinnamomi*. Some of the reasons that have been proposed are that clones of opposite mating type have different host preferences that limit contact, that they are genetically incompatible (or even of different ploidy), or that migrations bringing both mating types together have occurred too recently for the products of sexual reproduction to become detectable. Previous studies of *P. cinnamomi* in Australia identified only four multilocus isozyme genotypes among 445 isolates tested. These studies provide an ideal baseline for follow-up work to determine which kinds of changes have occurred during the past decade.

(viii) The true mating system of most *Phytophthora* species is undetermined. Although it is now known that homothallic species are capable of outcrossing, the fixation index values for most of them indicate that they do so only rarely in nature. Most of the heterothallic species had low fixation index values consistent with a high frequency of outcrossing. However, several exceptions were noted. Of particular interest are the high fixation index values for *P. capsici* and *P. botryosa*. Whether these result from close inbreeding (either from selfing or outcrossing among related individuals) or a Wahlund effect (due to combining of samples from different subpopulations) could be tested with additional sampling.

(ix) Finally, the apparently high heterozygosity for virulence compared to RFLP loci in *P. sojae*, if confirmed with additional data, must be explained. If there is overdominance at virulence loci (or chromosomal segments linked to them), it may occur in other *Phytophthora* species. This phenomenon has been well documented in plants but has not been studied in Oomycetes. Segregating progeny from selfed oospores could provide the biological materials needed to test this hypothesis.

These questions represent a sample of the many pressing questions concerning the population genetics of *Phytophthora* species. Most of these hypotheses make specific predictions and, thus, should be easy to test. If the current rapid pace of progress continues, many of the above questions will be answered during the next few years.

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