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Genetic Diversity and Origins of the Homoploid-Type Hybrid *Phytophthora ×alni*

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ABSTRACT

Assessing the process that gives rise to hybrid pathogens is central to understanding the evolution of emerging plant diseases. *Phytophthora ×alni*, a pathogen of alder, results from the homoploid hybridization of two related species, *Phytophthora uniformis* and *Phytophthora ×multiformis*. Describing the genetic characteristics of *P. ×alni* should help us understand how reproductive mechanisms and historical processes shaped the population structure of this emerging hybrid pathogen. The population genetic structure of *P. ×alni* and the relationship with its parental species were investigated using 12 microsatellites and one mitochondrial DNA (mtDNA) marker on a European collection of 379 isolates. Populations of *P. ×alni* were dominated by one multilocus genotype (MLG). The frequency of this dominant MLG increased after the disease emergence together with a decline in diversity, suggesting that it was favored by a genetic mechanism such as drift or selection. Combined microsatellite and mtDNA results confirmed that *P. ×alni* originated from multiple hybridization events that involved different genotypes of the progenitors. Our detailed analyses point to a geographic structure that mirrors that observed for *P. uniformis* in Europe. The study provides more insights on the contribution of *P. uniformis*, an invasive species in Europe, to the emergence of *Phytophthora*-induced alder decline.

IMPORTANCE

Our study describes an original approach to assess the population genetics of polyploid organisms using microsatellite markers. By studying the parental subgenomes present in the interspecific hybrid *P. ×alni*, we were able to assess the geographical and temporal structure of European populations of the hybrid, shedding new light on the evolution of an emerging plant pathogen. In turn, the study of the parental subgenomes permitted us to assess some genetic characteristics of the parental species of *P. ×alni*, *P. uniformis*, and *P. ×multiformis*, which are seldom sampled in nature. The subgenomes found in *P. ×alni* represent a picture of the "fossilized" diversity of the parental species.

Interspecific hybridization can be a rapid track for the evolution of new species in many phylogenetic groups, including fungi and oomycetes (1–3). It has been recognized as an important mechanism of plant disease emergence, with the description of many interspecific hybrid pathogens (4, 5). In particular, in the genus *Phytophthora*, interspecific hybridization is increasingly seen as a major force generating new taxa: hybrids have been successfully created under laboratory conditions, and more importantly, many natural hybrids have been reported (6–8). Thus, better knowledge of the processes that give rise to hybrid taxa is an important question in the evolutionary biology of emerging pathogens. Understanding the mechanisms that lead to speciation and to the establishment, spread, and evolution of hybrid taxa requires investigation of the identity and genetic variability of parental species and of the directionality and recurrence of the hybridization events (9, 10).

Analysis of allele and genotype frequencies using standard population genetic tools can at least in part answer these questions (11). However, the population genetics of polyploid organisms presents specific caveats: as allele copy numbers are ambiguous, computing allele frequencies and identifying multilocus genotypes can be complex (12). This has limited the use of these tools in the study of polyploid organisms. To tackle this problem, the development of specific primers from the contributing progenitor genomes may enable the analysis of population genetics of allopolyploids (13, 14). In this case, parental taxa must have been identified and their ploidy levels must be known. The identification of alleles from each parent and their separate treatment during the analysis can increase the robustness of the population genetic analysis (11).

The alder pathogen *Phytophthora ×alni* constitutes a good model to study the emergence and evolution of hybrids, since the parental taxa and their ploidy level are known. *Phytophthora uniformis* and *Phytophthora ×multiformis* have been identified as the parents of *P. ×alni* (15, 16). Recent work coupling flow cytometry and quantification of allele copy number determined the exact

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P.F. and B.M. codirected this work.

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ploidy levels of the three species of the complex (17). Accordingly, *P.* ×*alni* was shown to be a triploid homoploid-type taxon that formed through the hybridization of a diploid taxon (*P. uniformis*) and a tetraploid taxon (*P.* ×*multiformis*) without chromosome doubling. Therefore, *P.* ×*alni* possesses one-half of the genome of the two parental species.

P. uniformis, one of the parental species of *P.* ×*alni*, is a diploid species and has been isolated both in Europe and in North America (18–20). Genetic characterization of European *P. uniformis* populations revealed low diversity and a clonal structure, while North American populations were more diverse and exhibited signs of outcrossing (19). These results suggest that *P. uniformis* is exotic to Europe and that North America could be its center of origin. The introduction of *P. uniformis* to Europe would have enabled its hybridization with the other parental species and could thus be a major factor in the emergence of *P.* ×*alni*. Less is known about the origin of the other parental species, *P.* ×*multiformis*. This species is infrequently isolated, and only few individuals are available for analysis. Husson et al. (17) showed that *P.* ×*multiformis* is a tetraploid organism, supporting the hypothesis raised by Ioos et al. (15) that *P.* ×*multiformis* could have emerged after an ancient interspecific hybridization of two currently undetermined *Phytophthora* species. Nevertheless, whether *P.* ×*multiformis* and/or its parental species are exotic or indigenous to Europe remains unknown.

It was suggested that the spread of *P.* ×*alni* within Europe is the product of clonal dispersion (21, 22). The first evidence confronting this hypothesis came from the studies of Ioos et al. (15), who studied mitochondrial and nuclear molecular markers, which under sexual reproduction are inherited uniparentally and biparentally, respectively. The authors showed that *P.* ×*alni* isolates exhibit different mitochondrial (mtDNA) and nuclear DNA patterns that are shared either with *P. uniformis* (multiple U mitotypes for the mtDNA and different *P. uniformis* allele types for each of the nuclear DNA genes studied) or with *P.* ×*multiformis* (multiple M mitotypes for the mtDNA and different *P.* ×*multiformis* allele types for each of the nuclear DNA genes studied). These multiple-mitotype patterns suggest that several sexual hybridization events have led to the genesis of the hybrid and that the spread of *P.* ×*alni* throughout Europe might not be attributable to a single clone (15).

In this article, we analyzed further the role of sexual reproduction in the genesis and genetic diversification of the hybrid species *P.* ×*alni*. Based on 12 microsatellite markers and one mitochondrial DNA marker, we assessed the level of genetic diversity of *P.* ×*alni* in Europe and the manner in which this diversity is structured in a geographical and a temporal scale. This was done by studying the parental subgenomes of *P. uniformis* and *P.* ×*multiformis* occurring in *P.* ×*alni*. In turn, the study of these subgenomes allowed to assess some genetic characteristics of the parental species, *P. uniformis* and *P.* ×*multiformis*, which are seldom sampled in nature.

MATERIALS AND METHODS

Isolate collection and DNA extraction. For organisms with suspected low polymorphism, the choice of the sampling strategy is highly dependent on the objectives of the study. When the goal is to assess diversity in a population, as is the case here, the recommended strategy should be to sample along the whole distribution area of the target population, in order to attempt to collect more distinct and rare genotypes (23). This will minimize bias in the estimation of the diversity indices and deliver better approximations of the real population values. Here we also considered the temporal dimension. For this study, surveys of *Alnus glutinosa* and *Alnus incana* stands were conducted over 10 years (from 1999 to 2009) across France, Belgium, and Hungary to collect *P. uniformis*, *P.* ×*multiformis*, and *P.* ×*alni* samples. Sampling was performed from infected alder trees that exhibited typical *Phytophthora* collar canker symptoms. Small sections of necrotic bark pieces were placed on V8 juice or carrot agar medium selective for *Phytophthora*. As soon as detected, growing mycelium was transferred to fresh V8 juice agar containing rifampin (10 mg/liter) or nonamended carrot agar. To broaden sample collection, 57 additional isolates from 11 European countries were obtained from colleagues (see Table S1 in the supplemental material). Sampled locations are shown in Fig. 1. For *P. uniformis*, the European and North American isolates described by Aguayo et al. (19) were added as reference isolates to track the contribution of this invasive species to the emergence of *P.* ×*alni*. DNA extractions were performed from pure fresh or lyophilized mycelium using the BioSprint 96 DNA plant kit in combination with the BioSprint 96 automated workstation (Qiagen, Courtaboeuf, France), following the BS-96DNA-plant protocol, or using the Qiagen DNeasy Plant minikit according to supplier instructions. Isolate identity (*P.* ×*alni*, *P.* ×*multiformis*, or *P. uniformis*) was determined using the set of species-specific PCR primers TRP-PAU-F/-R, RAS-PAM1-F/-R, and RAS-PAM2-F/-R described by Husson et al. (17).

Study of the *P. alni* complex by microsatellite and mitochondrial markers. A set of microsatellite markers was generated independently from the two parental species. These markers were developed from microsatellite-enriched libraries sequenced with a Roche GS-FLX Titanium pyrosequencing platform following the procedure described by Malausa et al. (24) and Aguayo et al. (19). Markers obtained from the *P.* ×*multiformis* microsatellite-enriched library did not show polymorphism within any species of the *P. alni* complex; they were therefore discarded for further tests. Polymorphism was detected in *P.* ×*alni*, *P. uniformis*, and *P.* ×*multiformis* with markers obtained from the *P. uniformis* microsatellite-enriched library. This set of markers comprised five microsatellite markers developed by Aguayo et al. (19) and another five additional loci that exhibited polymorphism in *P.* ×*alni* and *P.* ×*multiformis*. Additionally, two polymorphic microsatellite loci derived from *P.* ×*alni*, PA17 and PA23, described by Ioos et al. (25), were added to the marker set. In all, we used 12 microsatellite markers to characterize our isolate collection. The PCR amplification conditions were those described by Aguayo et al. (19). The mitochondrial type of the *P.* ×*alni* isolates, either U or M mitotype (15), was studied using the *cox1* gene primers (COXF4N/COXR4N) designed by Kroon et al. (26) with PCR mix and run conditions as described by Ioos et al. (15). Subsequently, 20 μl of the PCR product was digested using HaeIII for 1 h at 37°C, according to the manufacturer's instructions (Invitrogen, Saint-Aubin, France). Digested DNA patterns were resolved after 1 h of electrophoresis (0.6 V/cm) in a 1% agarose gel in 0.5% Tris-borate-EDTA (TBE) buffer. Gels were stained with ethidium bromide to visualize polymorphisms of DNA fragments.

Assignment of microsatellite alleles to species of the *P. alni* complex. For each locus, we first assessed the different parental microsatellite profiles. As shown by Aguayo et al. (19), the diploid parent *P. uniformis* exhibited one or two alleles at all loci. For *P.* ×*multiformis*, we assumed the hypothesis of an ancient hybridization origin that formed a tetraploid hybrid as stated by Husson et al. (17). In the simplest cases, when one and four peaks were observed, we considered that each allele was present in four copies or one copy, respectively. If two or three peaks were observed, peak height ratios were used to assess allele copy numbers (27). In general, we observed two alleles with comparable peak sizes and we attributed two copies of each allele. Figure S1 in the supplemental material shows how chromatograms were used to determine the allele composition whenever 3 alleles where found in *P.* ×*multiformis*.

Microsatellite profiles for the triploid hybrid *P.* ×*alni* were then studied. First, at each locus we determined which specific alleles from each

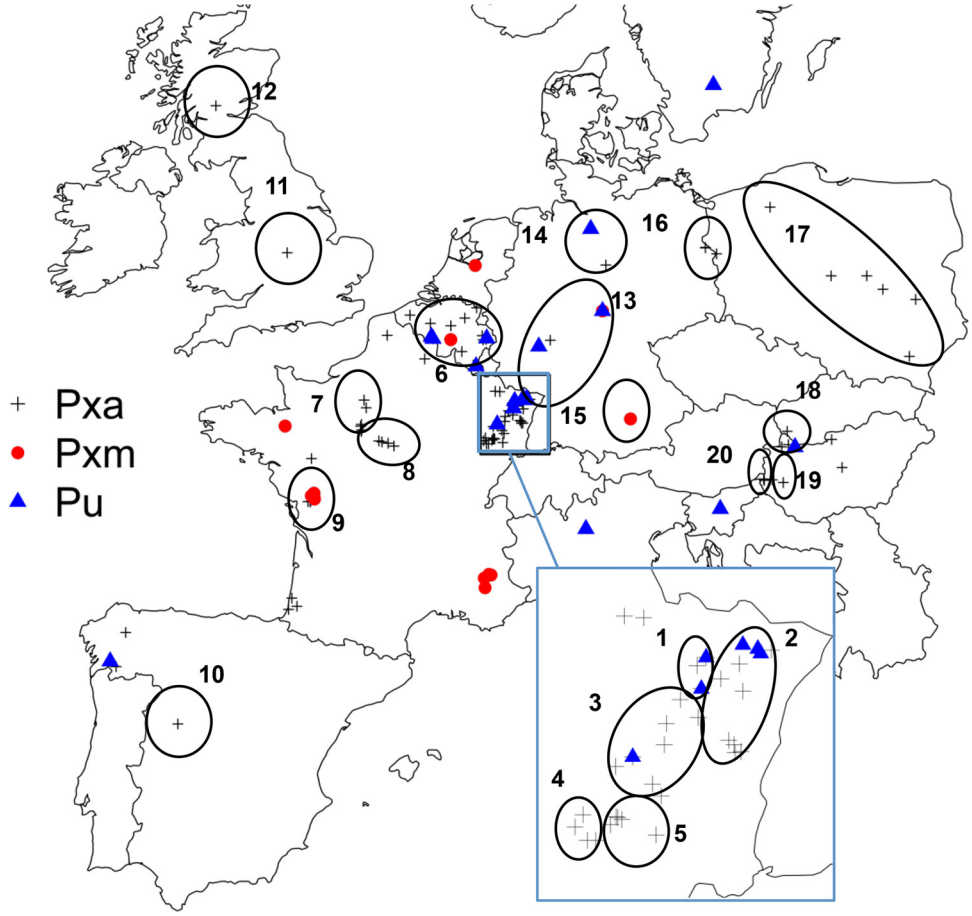

FIG 1 Geographical origins of *P. ×alni* (Pxa), *P. ×multiformis* (Pxm), and *P. uniformis* (Pu) isolates used in this study. 1, Sarre/Nied (France); 2, Rhine (France); 3, Meurthe/Moselle (France); 4, Saône (France); 5, Ognon (France); 6, Meuse (France and Belgium); 7, Loir (France); 8, Loire (France); 9, Sèvre Niortaise (France); 10, Salamanca (Spain); 11, England (United Kingdom); 12, Scotland (United Kingdom); 13, Rhineland (Germany); 14, Braunschweig (Germany); 15, Freising (Germany); 16, Oder (Germany); 17, Poland; 18, Répce (Hungary); 19, Balaton (Hungary); 20, Zala (Hungary).

parental species could be found in *P. ×alni*. When alleles present in *P. ×alni* were found exclusively in *P. uniformis* and not in *P. ×multiformis*, we assumed that they represented the haploid contribution of *P. uniformis* (here named *P. uniformis* subgenome [*Pu*SG]) to the genome of *P. ×alni*. When alleles in *P. ×alni* were found exclusively in *P. ×multiformis* and not in *P. uniformis*, we assumed that they represented the two haploid genomes' contribution of *P. ×multiformis* to *P. ×alni*, as described by Ioos et al. (15) and Husson et al. (17). The *P. ×multiformis* contribution will be referred to as *P. ×multiformis* subgenome (*P×m*SG) here. For the *P. ×multiformis* subgenome occurring in *P. ×alni*, different scenarios were considered: when only one *P. ×multiformis* allele was found in *P. ×alni*, these individuals were considered to be homozygous for the *P. ×multiformis* allele. Whenever two different *P. ×multiformis* alleles were found exclusively in *P. ×alni*, we considered that individuals might have inherited two different alleles coming from the double haploid contribution of *P. ×multiformis*. Another case occurred when *P. uniformis* and *P. ×multiformis* shared one allele. In such a case, we considered that the shared allele was a contribution of both *P. uniformis* and *P. ×multiformis* subgenomes in *P. ×alni*. In this case, the expected peak height ratio for the shared allele should be 2-fold compared to the remaining allele. The last case appeared when one allele was found exclusively in *P. ×alni* but not among parental isolates. In this case, alleles were assigned as belonging either to the *P. uniformis* or to the *P. ×multiformis* subgenome according to the mutation steps that separated the two subgenomes. To confirm reproducibility, a subset of 219 isolates (169 *P. ×alni*, 12 *P. uniformis*, and 38 *P. ×multiformis* isolates) were genotyped twice in independent PCR assays.

Characterization of the parental species *P. uniformis* and *P. ×multiformis* and comparisons with reconstructed subgenomes in *P. ×alni*. Microsatellite profiles of *P. uniformis* and *P. ×multiformis* were compared to the reconstituted subgenomes in *P. ×alni*. Each locus in *P. ×alni* was converted into diploid profiles for the *P. uniformis* subgenome and to tetraploid profiles for the *P. ×multiformis* subgenome. The *P. uniformis* subgenome was compared to *P. uniformis* North American and European isolates, and the *P. ×multiformis* subgenome was compared to European *P. ×multiformis* samples. Multilocus genotypes (MLGs) were identified using Genotype (28), computing distances under the stepwise mutation model (SMM). The assignment was performed by computing a pairwise distance matrix and selecting a threshold that defines the maximum distance between two individuals at which they are still assigned to the same clonal lineage. The threshold was set at zero to differentiate individuals with small differences. Genotype can handle putative missing alleles by comparing the apparent difference in ploidy level in one locus to other loci of the same individual. Whenever an allele was missing due to potential amplification problems, it was considered a null allele. Poppr (29) was used to assess genotypic richness, genotypic diversity, and the evenness for each group of samples. Genotypic richness was assessed by computing the raw number of observed MLGs and the expected MLG (eMLG) corrected by the smallest sample size based on rarefaction. Genotypic diversity was assessed with the Simpson's diversity index (*D*), corrected by sample size

across all samples. *D*, defined as the probability that two individuals taken at random belong to different MLGs, was equation 1 computed as

$$\left(\frac{N}{N-1}\right)\left(1-\sum_{i=1}^{G} p_i^2\right) \quad (1)$$

where N is the sample size, G the number of MLGs detected over all samples, and p_i is the frequency of the ith MLG in the sample. Evenness (E_5), an index that reflects equitability in the distribution of clonal membership among samples, was also estimated. E_5 is the ratio of the number of abundant genotypes to the number of rarer genotypes (30). Evenness may range from 0 to 1, with 0 for a population dominated by a single MLG, and 1 for a population with equally abundant MLGs. Clonal richness (R), i.e., the ratio of the number of MLGs found over the sample size, was computed according to the formula $(G-1)/(N-1)$. R ranges from 0, when all individuals share the same MLG, to 1, when all individuals have distinct MLGs. Minimum spanning networks (MSN) were constructed to assess genetic relationships among subgenomes and parental genomes. Poppr was used to compute the Bruvo pairwise genetic distance (31) between samples of the *P. uniformis* subgenome and *P. uniformis* and of the *P.* ×*multiformis* subgenome and *P.* ×*multiformis*. The Bruvo distance, adapted to polyploid organisms, is similar to band-sharing indices used with dominant data but takes into account the stepwise mutational distance between microsatellite alleles (12). The distance ranges from 0, indicating identical MLGs, to 1, indicating maximum dissimilarity. This distance is better adapted to autopolyploids but can be applied to allopolyploids, provided that when alleles present at one locus can originate from the two subgenomes, these alleles are separated by at least three mutation steps (32).

Last, we compared *P. uniformis* and *P.* ×*multiformis* subgenome profiles to the observed MLGs of the parental isolates to decipher their *in natura* contribution to the variability of *P.* ×*alni*. Simulated crosses between all parental *P. uniformis* and *P.* ×*multiformis* MLGs were performed under the hypothesis of a sexual origin of the hybrid to determine whether some of the possible mating combinations could generate a specific *P.* ×*alni* MLG. When a perfect match was found, the parental *P. uniformis* and *P.* ×*multiformis* MLGs were considered putative progenitors of *P.* ×*alni*.

Geographical and temporal structure of *P.* ×*alni*. For the whole *P.* ×*alni* collection (269 samples), MLGs were identified using Genotype as explained previously. Genotypic richness, genotypic diversity, and evenness were computed using Poppr. Clonal richness corrected by sample size was also estimated. Two approaches were used to study the geographical population structure of *P.* ×*alni*. The first approach used principal-component analysis (PCA) to assess genetic relationships among samples of *P.* ×*alni*. Multivariate analyses such as PCA are exploratory methods that can be used to cluster individuals without making strong assumptions about an underlying genetic model, allowing to summarize genetic variability to reveal structure (33). POLYSAT (12) was used to compute the Bruvo distance between *P.* ×*alni* samples. The distance matrix was visualized after scaling using PCA with FactoMineR (34). An analysis of variance (ANOVA) test was used to assess whether *P.* ×*alni* clusters observed in PCA could be explained by the geographical coordinates and by the putative *P. uniformis* and *P.* ×*multiformis* parents assigned to *P.* ×*alni* after the simulated sexual crosses. Likewise, the homogeneity of mtDNA type (M or U) distribution in Europe was tested by ANOVA, using the geographical coordinates of each sampling location as independent variables.

The second approach used conventional population genetics tools based on allele frequency. For this approach, isolates were assigned to 20 watershed/country populations. The number of isolates per site was in many cases limited because of low isolation rates in some locations. We thus grouped isolates at a larger geographical scale (minimum group size of 4). As *P.* ×*alni* is able to disperse over long distances by river water, isolates were grouped by watershed whenever the exact sampling locations were known. Seventeen watershed populations could be defined (see Table 3). Three additional populations (from England, Scotland, and Poland) were also defined for isolates with unknown or incomplete geographical coordinates. These three populations were arbitrarily located at the country's barycenter. The number of *P.* ×*alni* isolates that could be assigned to watershed/country populations (populations with at least four *P.* ×*alni* individuals) was 254. The remaining 15 isolates could not be assigned to a watershed or a country population because it was not possible to group more than 4 isolates together. For each population, we computed the number of observed MLGs, clonal richness, and genotypic diversity as described previously. Population differentiation was studied by estimation of the genetic differentiation indexes *F*st and *R*st, using SPAGeDi 1.3a (35). The *F*st and *R*st indexes measure the apportionment of genetic variability between population and individual levels, based on allelic states and allele sizes, respectively. Polyploids with disomic inheritance, such as allopolyploids, can be analyzed using these two estimators when alleles from the different subgenomes can be distinguished in the hybrid genome. Both indexes were tested for significance by 10,000 permutations. Diversity indexes and genetic differentiation were assessed for *P.* ×*alni* isolates and for their reconstructed *P. uniformis* and *P.* ×*multiformis* subgenomes for each population.

For the temporal structure of *P.* ×*alni*, the evolution of clonal richness over the years was compared by regression with a linear model. To account for the strong unbalanced sampling between years and locations, we simulated samples by randomly selecting one isolate per year for each location. Estimation of the mean clonal richness per year was computed after 1,000 repetitions. By using the same procedure, we studied the evolution of the mtDNA type, either M or U, over the years. Temporal population differentiation of *P.* ×*alni* was assessed by estimation of global *F*st and *R*st using SPAGeDi 1.3a as described above for geographical structure. Collection years were known for 266 *P.* ×*alni* samples, which were used to determine temporal structure.

RESULTS

Microsatellite analyses and allele assignment to *P. uniformis* and *P.* ×*multiformis* subgenomes. A total of 379 isolates were genotyped (269 *P.* ×*alni*, 39 *P.* ×*multiformis*, and 71 *P. uniformis* isolates), including all the 71 *P. uniformis* isolates previously studied by Aguayo et al. (19). Repeatability of the allele peak height ratios was consistent between replicates ($R^2 = 0.76$; $P < 0.001$). This information was therefore used to estimate the allele copy number present in each isolate whenever the number of alleles was lower than expected and to assign alleles to *P. uniformis* and *P.* ×*multiformis* subgenomes. Polymorphism of the microsatellite markers was low, although all loci exhibited polymorphism for at least one species of the *P. alni* complex. In total, 34 alleles were amplified for the 12 microsatellite loci. The number of alleles at each locus for each species ranged from 1 to 4 (Table 1). Consistent with the hypothesis used for ploidy levels, 1 or 2, 1 to 4, and 1 to 3 alleles per isolate were amplified at each locus in *P. uniformis*, *P.* ×*multiformis*, and *P.* ×*alni*, respectively. Four microsatellite loci amplified exclusively in *P. uniformis* and *P.* ×*alni*, and one exclusively in *P.* ×*multiformis* and *P.* ×*alni* (Table 1). The Venn diagram in Fig. 2 displays the allelic distribution within the three species. Most alleles were specific either to both *P. uniformis* and *P.* ×*alni* or to both *P.* ×*multiformis* and *P.* ×*alni*. Only one allele was shared among the three species (allele 68 at locus M-PAU14).

Concerning the parental species *P. uniformis*, 4 private alleles were observed. Three were observed only in North American samples (allele 90 at locus M-PAU32, allele 95 at locus M-PAU9, and allele 202 at locus M-PAU53), while one was shared by American and European samples (allele 105 at locus M-PAU3). No private *P.* ×*multiformis* alleles were observed. Concerning *P.* ×*alni*, all alleles could be assigned either to a *P. uniformis* or to a *P.* ×*multiformis* subgenome (Table 2). One single allele was found at each

TABLE 1 Characteristics of the microsatellite markers used for this study

Locus	GenBank accession no.	Primer[d] sequence (5′–3′)	Repeat motif	Size (bp)	Allele(s)		
					P. uniformis	*P. ×multiformis*	*P. ×alni*
M-PAU3[a]	JX462795	F, TAAGAGACCTCCGGCAGAGA R, AAAGCGAACACGAAGTCCAC	$(GA)_{10}$	110	105/107/113		107/113
M-PAU9[a]	JX462796	F, TCATGGCGCTGATCAAGTAG R, TAGTGGAGACTTACGGGGTT	$(AC)_9$	95	93/95	83	83/93
M-PAU11[c]	JX462790	F, AGGTGGAGTGCTAGAGGCAA R, GCGACCTTTGAGTGACCAAT	$(CAT)_7$, $C(TTC)_9$	189	151	188/191	151/185/188/191
M-PAU14[c]	JX462791	F, GAAGGCTACGTAACTTGCTTTT R, ATCGAACTTCTCTTCCTTCACG	$(GT)_9$	80	68	62/68/75/77	62/68/75/77
M-PAU15[c]	JX462792	F, CCCGTCCTTCATCAACAAAA R, GAGGCTCTGCGATGCAATAG	$(CT)_9$	80	74	71	71/74
M-PAU32[a]	JX462797	F, TCAGCTCCTGTATCATCAATCG R, AAGTTGCCGGTGAGTTGG	$(CA)_9$	99	90/92	86	86/92
M-PAU53[a]	JX462798	F, TCTGACGAAGACCTCGACCT R, CTCGAGATTGCCTTGCTGTC	$(CT)_8$	187	202/204		204/206
M-PAU55[a]	JX462799	F, ACATTGCTCATTCAGATGCG R, GTGGAGGAGCACTTCATGGT	$(GT)_8$	232	243/245		243/245
M-PAU56[c]	JX462793	F, GCTGGTGGATAATTCGTCGT R, CAAAAGCGATCCTCTTCACC	$(GA)_7$	81	71	74/78	71/74/78
M-PAU72[c]	JX462794	F, GTTCTCGAGACTCAGCAGCC R, CAGAGGGATACCCGAGTGAA	$(CAA)_7$	146	140	152/156	140/152/156
PA17[b]	DQ665905	F, AGCGACAATGCAGGAAGC R, CTGTCTGGGCATTCATGTCG	$(GTC)_4(\ldots)(GC)_4$	317	317		317/322
PA23[b]	DQ665906	F, GGAGATAGCCACGAGACACC R, CAAGCATCGCTGTAAACGAC	$(GAA)_7$	155		135/150	135/150

[a] From Aguayo et al. (19).
[b] From Ioos et al. (25).
[c] From this study.
[d] F, forward; R, reverse.

exclusive *P. uniformis* and *P. ×alni* locus. For these loci, the observed allele was thus considered to represent the haploid contribution of the *P. uniformis* subgenome. Similarly, 1 or 2 alleles were present in *P. ×alni* at loci exclusive to *P. ×multiformis* and *P. ×alni*, and they were considered to represent the haploid contribution of the *P. ×multiformis* subgenome. For loci that cross-amplified in the three species, assignment was usually straightforward since they generally differed between *P. uniformis* and *P. ×multiformis*. The only shared allele (allele 68 at locus M-PAU14) was found in all *P. ×alni* samples. For 99% of the *P. ×alni* isolates that presented 3 alleles at locus M-PAU14, the alleles exhibited similar height ratios, and assignment was straightforward. For the 1% of the *P. ×alni* isolates that exhibited only two alleles at locus M-PAU14, peak height ratios enabled us to determine that allele 68 was present in two copies, and it was assumed that one copy originated from *P. uniformis* and the other from *P. ×multiformis*. Three private alleles were observed exclusively in *P. ×alni*, though present in few individuals. These alleles were at one mutation step away from their putative parental alleles in *P. ×multiformis* (allele 185 at locus M-PAU11) and *P. uniformis* (allele 206 at locus M-PAU53 and allele 322 at locus PA17). Eleven *P. ×alni* isolates (4.09%) presented missing alleles at least at one locus, which were considered null alleles. It is interesting that private *P. uniformis* alleles observed only in North American samples were very seldom observed in *P. ×alni*. The only exception was allele 243 at locus M-PAU55, which was observed in one Hungarian isolate. Multilocus allelic profiles for all isolates used in this study are presented in Table S1 in the supplemental material.

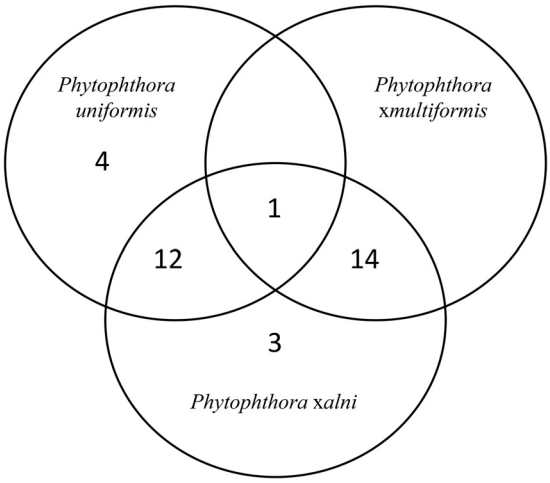

FIG 2 Venn diagram representing the allelic distribution within *P. ×alni*, *P. ×multiformis*, and *P. uniformis*. Numbers in the circles represent the number of alleles found in one specific species or in more than one of the species simultaneously.

Relationship between *P. ×alni* and parental species *P. uniformis* and *P. ×multiformis*. Seven MLGs were observed in North American *P. uniformis* samples. Although clonal richness was low ($R = 0.23$), genotypic diversity ($D = 0.70$) and evenness ($E_5 = 0.63$) were moderate. Diversity in European *P. uniformis* was low, with only 3 observed MLGs. One dominant MLG (*Pu*-E1) included 91% of the isolates (see Table S1 in the supplemental ma-

TABLE 2 Multilocus genotypes of *P. ×alni* and their putative parents after computed mating simulations[a]

P. ×alni MLG	*P. uniformis* subgenome MLG	*P. ×multiformis* subgenome MLG	*n*	*P. uniformis* putative parents[b]	*P. ×multiformis* putative parents	Cluster
P×a-1	*Pu*-SG1	*P×m*-SG1	216	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2	A
P×a-2	*Pu*-SG3	*P×m*-SG1	10	*Pu*-E2	*P×m*-1, *P×m*2	B
P×a-3	*Pu*-SG3	*P×m*-SG7	7	*Pu*-E2	*P×m*-2, *P×m*-4	B
P×a-4	*Pu*-SG1	*P×m*-SG9	4	*Pu*-E1, *Pu*-E3	nf	A
P×a-5	*Pu*-SG1	*P×m*-SG4	3	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2	A
P×a-6	*Pu*-SG1	*P×m*-SG5	3	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2, *P×m*-5	A
P×a-7	*Pu*-SG1	*P×m*-SG6	3	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2	A
P×a-8	*Pu*-SG1	*P×m*-SG2	3	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2, *P×m*-5	A
P×a-9	*Pu*-SG1	*P×m*-SG7	3	*Pu*-E1, *Pu*-E3	*P×m*-2, *P×m*-4	A
P×a-10	*Pu*-SG1	*P×m*-SG2	1	nf[c]	nf	C
P×a-11	*Pu*-SG1	*P×m*-SG3	1	*Pu*-E1, *Pu*-E3	*P×m*-2, *P×m*-4	A
P×a-12	*Pu*-SG2	*P×m*-SG6	1	nf	*P×m*-1, *P×m*-2	C
P×a-13	*Pu*-SG2	*P×m*-SG1	1	nf	*P×m*-1, *P×m*-2	A
P×a-14	*Pu*-SG3	*P×m*-SG8	1	*Pu*-E2	*P×m*-2, *P×m*-4	B
P×a-15	*Pu*-SG3	*P×m*-SG5	1	*Pu*-E2	*P×m*-1, *P×m*-2, *P×m*-5	B
P×a-16	*Pu*-SG4	*P×m*-SG1	1	nf	*P×m*-1, *P×m*-2	A
P×a-17	*Pu*-SG3	*P×m*-SG1	1	*Pu*-E2	*P×m*-1, *P×m*-2	B
P×a-18	*Pu*-SG3	*P×m*-SG10	1	*Pu*-E2	*P×m*-3	B
P×a-19	*Pu*-SG1	*P×m*-SG11	1	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2, *P×m*-5	A
P×a-20	*Pu*-SG1	*P×m*-SG8	1	*Pu*-E1, *Pu*-E3	*P×m*-2, *P×m*-4	A
P×a-21	*Pu*-SG1	*P×m*-SG1	1	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2	A
P×a-22	*Pu*-SG1	*P×m*-SG12	1	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2	A
P×a-23	*Pu*-SG5	*P×m*-SG1	1	nf	*P×m*-1, *P×m*-2	A
P×a-24	*Pu*-SG1	*P×m*-SG1	1	nf	*P×m*-1, *P×m*-2	A
P×a-25	*Pu*-SG1	*P×m*-SG6	1	nf	nf	C
P×a-26	*Pu*-SG1	*P×m*-SG13	1	*Pu*-E1, *Pu*-E3	*P×m*-1, *P×m*-2	A

[a] Complete data are presented in Table S1 in the supplemental material.
[b] *P. uniformis* multilocus genotypes are those described by Aguayo et al. (19) but renamed according to Husson et al. (17).
[c] nf, no putative parent could be found.

terial). This influenced the low levels of clonal richness ($R = 0.05$), genotypic diversity ($D = 0.18$), and evenness ($E_5 = 0.14$) exhibited by European isolates. The number of expected MLGs (eMLG) at the smallest sample size (27 North American isolates) based on rarefaction was 2.58. Concerning the *P. uniformis* subgenome, 5 MLGs were observed. Two MLGs were shared between European *P. uniformis* isolates and the *P. uniformis* subgenome (*Pu*-E1 and *Pu*SG-1 and *Pu*-E2 and *Pu*SG-3). As for *P. uniformis* isolates, a dominant *P. uniformis* subgenome MLG (*Pu*SG-1) grouped 91% of the isolates. The eMLG index for the *P. uniformis* subgenome data set was 2.2, a value close to the one observed for the European *P. uniformis* isolates, assuming equal sample sizes. Similar to what was seen for European *P. uniformis*, the *P. uniformis* subgenome data set presented low levels of clonal richness ($R = 0.01$), genotypic diversity ($D = 0.17$), and evenness ($E_5 = 0.14$). The minimum spanning network (MSN) clearly differentiated two groups of isolates (Fig. 3a). A first group included exclusively North American *P. uniformis* isolates (MLGs *Pu*-A1 to *Pu*-A7). A second group included European *P. uniformis* isolates and *P. uniformis* subgenome MLGs, indicating their straight relationship. Within this group, *Pu*SG-1 MLG was placed together with *Pu*-E1 and *Pu*-E3 MLGs, which were found as its putative parents after simulated crosses (see Table S1 in the supplemental material). Similarly, *Pu*SG-3 was placed together with *Pu*-E2, its putative *P. uniformis* parent (see Table S1 in the supplemental material). *Pu*SG-2, *Pu*SG-4, and *Pu*SG-5 grouped 2.6% of the isolates characterized by nonsampled *P. uniformis* putative parents.

Five MLGs were identified among *P. ×multiformis* isolates, with one major MLG (*P×m*-1) including 56% of the isolates. *P. ×multiformis* clonal richness was low ($R = 0.11$), and genotypic diversity was moderate ($D = 0.59$). The evenness index was estimated at 0.72, indicating some dissimilarity among samples. For the *P. ×multiformis* subgenome, 13 MLGs were observed, with clonal richness and genotypic diversity levels of 0.04 and 0.25, respectively. Estimated *P. ×multiformis* subgenome eMLG rarefied to 39 samples was 5.12, indicating that genotypic richness was similar to that of *P. ×multiformis* when considering equal samples. MSN did not exhibit a clear structure pattern because polymorphism was low and several *P. ×multiformis* subgenome MLGs shared putative parents (Fig. 3b). This is specially the case for putative parents *P×m*-1 and *P×m*-2, which were found as putative progenitors for 89% of *P. ×multiformis* subgenome MLGs (Table 3; see also Table S1 in the supplemental material). The percentage of isolates for which putative progenitors could not be attributed was 2.6%.

General genetic characteristics of *P. ×alni*. Twenty-six MLGs were observed in *P. ×alni* samples. One dominant MLG (*P×a*-1) included 80% of the isolates. Minor MLGs, comprising 3 to 10 isolates, encompassed 13% of the isolates. Seventeen MLGs (6% of the isolates) were sampled once. Clonal richness was low ($R = 0.09$), and genotypic diversity was moderate ($D = 0.35$). Evenness was low ($E_5 = 0.288$), indicating low diversity within *P. ×alni* isolates. Mitochondrial types studied for 265 *P. ×alni* isolates showed that most (80%) had an M mtDNA profile while 20% exhibited a U mtDNA. In four microsatellite MLGs, both U and M mitotypes were present, adding a new level of polymorphism.

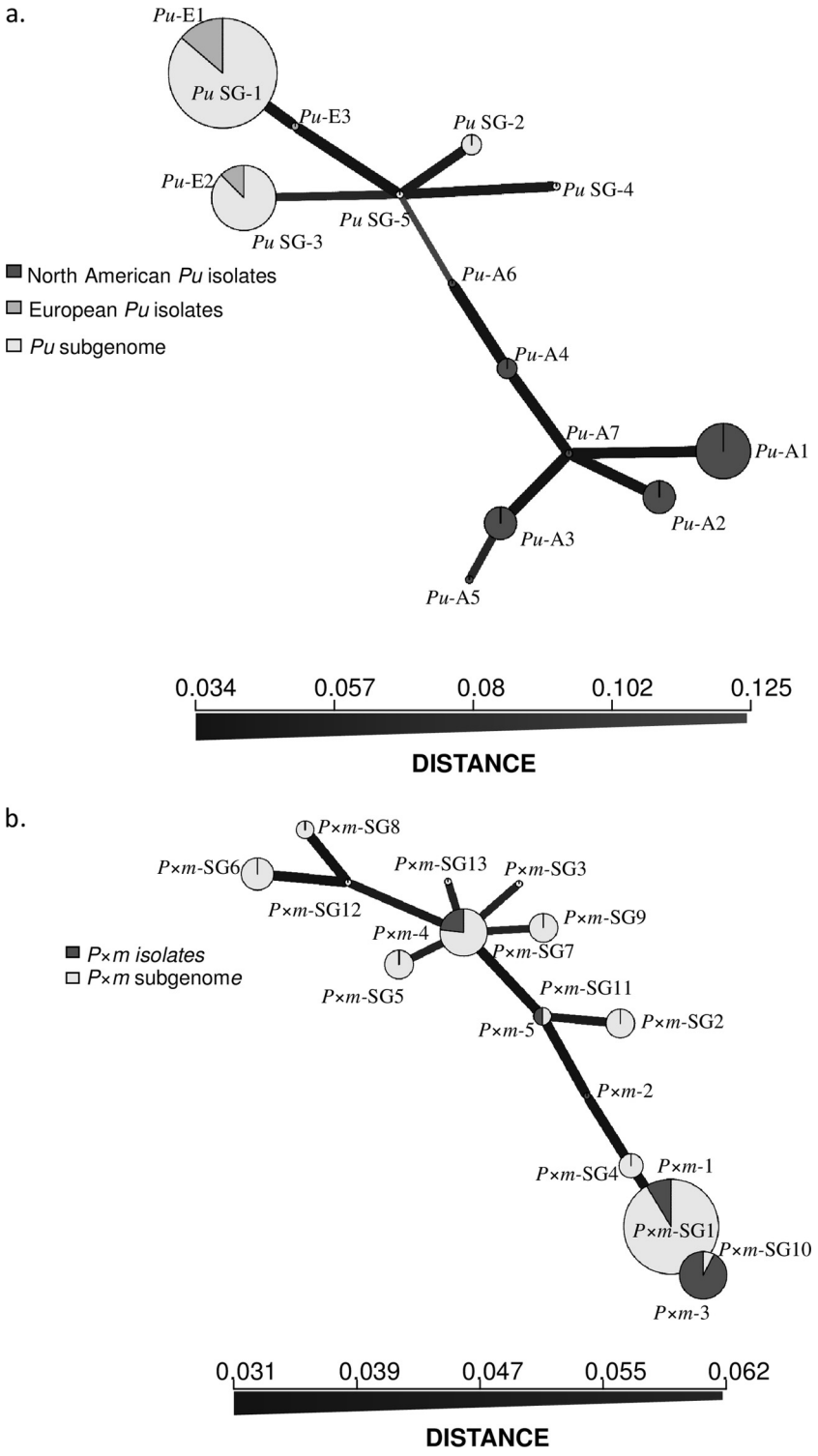

FIG 3 Minimum spanning network (MSN) performed on Bruvo's distances. (a) *Phytophthora uniformis* isolates (*Pu*) and *P. uniformis* subgenome (*Pu*SG) found in *P.* ×*alni* isolates. (b) *Phytophthora* ×*multiformis* isolates (*P*×*m*) and *P.* ×*multiformis* subgenome (*P*×mSG) found in *P.* × *alni* isolates.

When microsatellite and mtDNA data were combined, the number of MLGs increased to 30. This means that microsatellite MLGs with both mtDNAs are not true lineages but the product of at least two hybridization events. Under a conservative estimation (considering only known assigned parents to *P.* ×*alni* MLGs), the number of hybridization events was at least 13.

Geographical structure of *P.* ×*alni* populations. The conditions required to use Bruvo's distance for *P.* ×*alni* were fulfilled,

TABLE 3 Characteristics of *P.* ×*alni* populations in Europe

ID no.	Country	Population	No. of samples	M/U type[a]	*P.* ×*alni*			*Pu*SG			*P*×*m*SG		
					MLG	*R*	*D*	MLG	*R*	*D*	MLG	*R*	*D*
1	France	Sarre/Nied	37	37/0	4	0.08	0.16	2	0.03	0.05	3	0.06	0.11
2	France	Rhine	10	9/1	2	0.11	0.2	1	0	0	2	0.11	0.2
3	France	Meurthe/Moselle	8	6/2	2	0.14	0.25	1	0	0	2	0.14	0.25
4	France	Saône	21	20/1	2	0.05	0.1	1	0	0	1	0	0
5	France	Ognon	5	4/1	2	0.25	0.4	1	0	0	2	0.25	0.4
6	France-Belgium	Meuse	19	17/2	1	0	0	1	0	0	1	0	0
7	France	Loir	12	0/10 (2 NA)	1	0	0	1	0	0	1	0	0
8	France	Loire	8	5/3	2	0.14	0.25	1	0	0	2	0.14	0.25
9	France	Sèvre Niortaise	14	13/1	1	0	0	1	0	0	1	0	0
10	Spain	Salamanca	6	6/0	1	0	0	1	0	0	1	0	0
11	United Kingdom	England	5	3/2	4	0.75	0.9	2	0.25	0.4	3	0.5	0.8
12	United Kingdom	Scotland	4	3/1	1	0	0	2	0	0	1	0	0
13	Germany	Rhineland	6	5/0 (1 NA)	4	0.6	0.87	1	0	0	4	0.6	0.87
14	Germany	Braunschweig	4	2/2	4	1	1	3	0.67	0.83	3	0.67	0.83
15	Germany	Freising	6	0/6	1	0	0	1	0	0	1	0	0
16	Germany	Oder	9	9/0	3	0.25	0.42	1	0	0	3	0.25	0.42
17	Poland	Poland	9	2/7	2	0.13	0.5	2	0.13	0.5	1	0	0
18	Hungary	Répce	26	19/7	6	0.2	0.65	3	0.08	0.52	3	0.08	0.22
19	Hungary	Balaton	9	8/1	2	0.13	0.22	1	0	0	2	0.13	0.22
20	Hungary	Zala	36	36/0	3	0.06	0.11	3	0.06	0.11	2	0.03	0.06

[a] Abbreviations: M/U type, frequency of samples with M or U mtDNA type; NA, information not available; MLG, number of multilocus genotypes per population; *R*, clonal richness; *D*, genotypic diversity (Simpson's index); *Pu*SG, *P. uniformis* subgenome in *P.* ×*alni* isolates; *P*×*m*SG, *P.* ×*multiformis* subgenome in *P.* ×*alni* isolates.

except for allele 68 at locus M-PAU14, which was shared by the two parental species. However, this allele was present in all *P.* ×*alni* samples and did not impact the estimation of genetic distances between isolates. The first two axes of the PCA explained 98.5% of the total variation and revealed a strong structure with three distinct clusters (Fig. 4). A first cluster, here called A, grouped most of the samples (91% of the isolates and 17 of the 26 *P.* ×*alni* MLGs). Consistently with the MSN built for *P. uniformis* and *Pu*SG, cluster A was characterized by isolates that had either *Pu*-E1, *Pu*-E3, or a nonsampled MLG as putative *P. uniformis* parent. A second cluster, here called B, grouped samples with the *Pu*-E2 MLG as the putative *P. uniformis* parent. The third cluster (C) is less clear, was characterized by samples with missing alleles at some loci, and therefore had nonsampled *P. uniformis* putative parents. As for the MSN computed for *P.* ×*multiformis* and *P*×*m*SG, the *P.* ×*multiformis* putative parents did not show any relationship with the structure of the *P.* ×*alni* clusters. Significant differences were found between samples of the different clusters when tested against latitude ($F = 16.6$; $P < 0.001$) and longitude ($F = 12.8$; $P < 0.001$), with clusters B and C located further east and north than cluster A (Fig. 5). Neither latitude nor longitude was found to be significantly related to the mtDNA type of samples ($F = 0.07$, $P = 0.78$; and $F = 3.74$, $P = 0.053$, respectively).

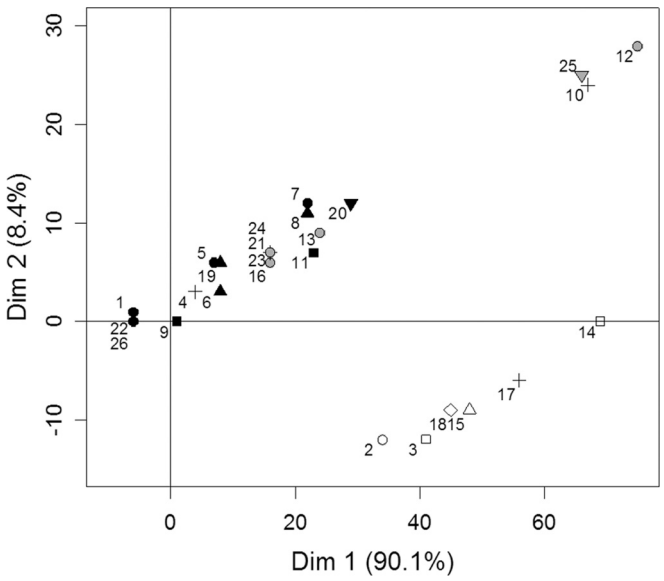

FIG 4 Principal component analysis (PCA) performed on Bruvo's distances on the whole collection of *P.* ×*alni* (269 isolates). Symbols indicate *P.* ×*multiformis* and *P. uniformis* putative parents: ○, *P*×*m*-1 or *P*×*m*-2; □, *P*×*m*-2 or *P*×*m*-4; △, *P*×*m*-1, *P*×*m*-2, or *P*×*m*-5; ▽, *P*×*m*-2; ◇, *P*×*m*-4; +, undetermined. Black filled symbols, *Pu*-E1 or *Pu*-E3; empty symbols, *Pu*-E2; gray filled symbols, undetermined *P. uniformis* putative parents. The numbers indicate the *P.* ×*alni* MLGs.

Diversity and population structure were further studied for 254 samples that could be assigned to the 20 watershed/country populations. Diversity indices for each population are listed in Table 3. No genetic diversity was found for six populations (Freising, Loir, Sèvre Niortaise, Meuse, Salamanca, and Scotland), and only the *P*×*a*-1 MLG was present. In contrast, the Braunschweig population exhibited different MLGs for the 4 sampled isolates. Although limited in size, the England and Rhineland populations exhibited relatively high diversity, with levels of clonal richness and genotypic diversity close to 1. Moderate diversity indices were estimated for the Oder, Ognon, and Répce populations. For the remaining populations, diversity indices were low. In most populations, the dominant *P*×*a*-1 MLG was present in frequencies ranging from 0.20 to 1, except in the Oder population, where *P*×*a*-1 was absent. *P*×*a*-3 was observed only in the Oder population, while *P*×*a*-4 was observed only in the Ognon population.

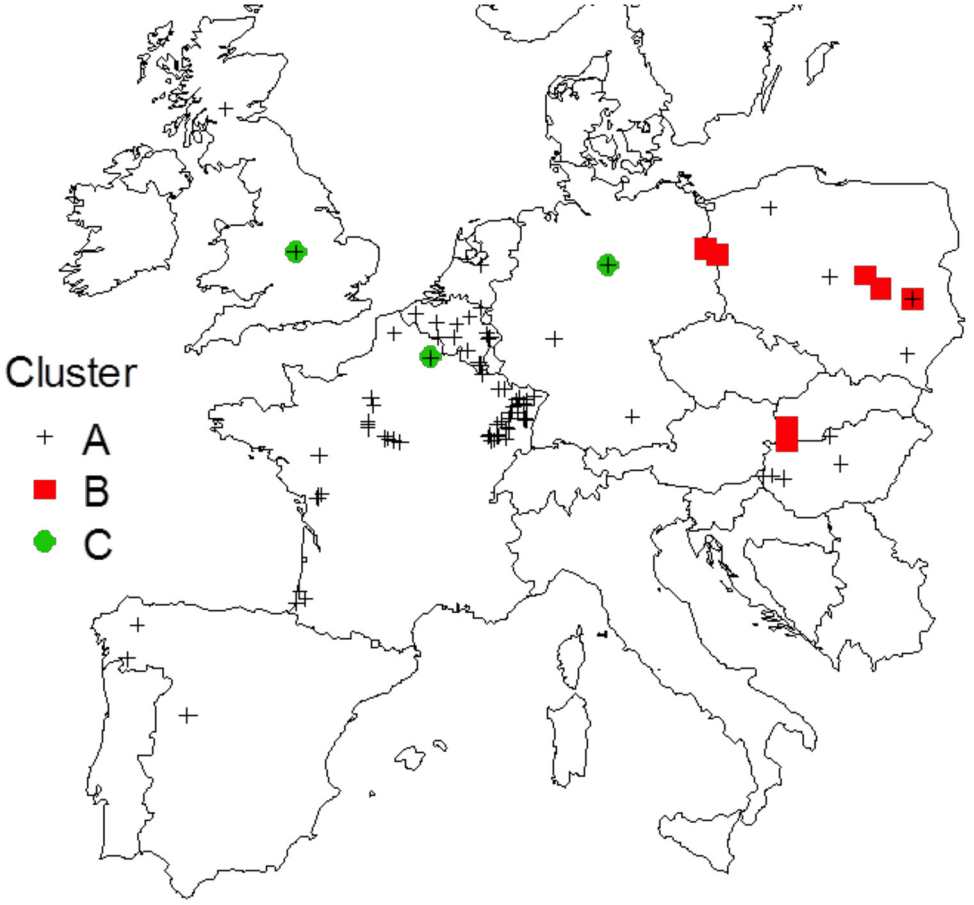

FIG 5 Geographical distribution of *P.* ×*alni* clusters determined by principal component analysis (PCA). Isolates from clusters B and C are distributed mostly in the north and the east of Europe, in contrast to isolates from cluster A.

Rare MLGs (only one isolate) were observed in England, Braunschweig, Oder, Répce, Rhin, Rhineland, Saône, Sarre and Nied, and Zala populations. It must be taken into account that three rare MLGs (*P*×*a*-24, *P*×*a*-25, and *P*×*a*-26) were not included in this section of the study because these isolates could not be assigned to any watershed/country population. The *P.* ×*alni* populations presented low, albeit significant, genetic differentiation, with estimated *F*st of 0.024 ($P < 0.001$) and *R*st of 0.004 ($P < 0.001$). Genetic diversity within populations was significantly lower for the *P. uniformis* subgenome than for the *P.* ×*multiformis* subgenome. Indeed, no genetic diversity was found for 14 of 20 populations when they were analyzed for the *P. uniformis* subgenome. Despite this low population diversity, when evaluated for the *P. uniformis* subgenome, strong genetic differentiation was found with high *F*st and *R*st values (*Fst* = 0.51, $P < 0.001$; *Rst* = 0.56, $P < 0.001$). When populations were analyzed for the *P.* ×*multiformis* subgenome, genetic differentiation estimates were similar to those obtained for *P.* ×*alni*, with low but significant *F*st and *R*st (*Fst* = 0.02, $P < 0.001$; *Rst* = 0.021, $P < 0.001$).

Temporal structure of *P.* ×*alni* isolates. Figure 6 summarizes the assessment of the temporal changes of the clonal richness for *P.* ×*alni*. The regression showed a significant decrease of the clonal richness from 1996 to 2010 ($F = 12.1$, $R^2 = 0.559$, $P = 0.013$). *P*×*a*-1 had little presence at the beginning of the sampling period, and was strongly dominant in the late collection years

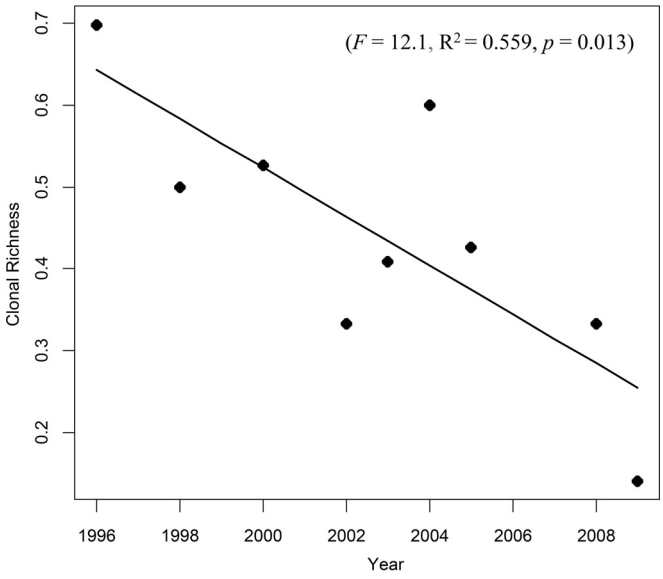

FIG 6 Temporal changes of clonality in *P.* ×*alni*. Linear regression shows that clonal richness decreased with time.

(after 2000). Changes in the ratio of the mtDNA haplotypes, M or U, over the years did not show any significant trend, staying stable during the evaluated period ($P = 0.07$). Moreover, while samples of clusters A and B were collected at about the same time (respective means of 2006 ± 0.5 and 2007 ± 1.1), samples of cluster C were collected much earlier (1999 ± 3.9). *F*st and *R*st estimated values suggest a low but significantly different from zero temporal differentiation (*F*st = 0.003, $P = 0.04$; and *R*st = 0.001, $P = 0.003$).

DISCUSSION

In our study, we were able to assign alleles from parental subgenomes to most *P.* ×*alni* samples by combining the analysis of microsatellite profiles and peak height ratios. Microsatellites revealed low polymorphism in *P.* ×*alni*. Yet, the genotypic richness and diversity within *P.* ×*alni* were consistent with those of its parental species. Moreover, our approach allowed us to explore the diversity of both parental species, by studying the extra genetic variability that is found in *P.* ×*alni* subgenomes. In the case of *P. uniformis* and *P.* ×*multiformis*, this is interesting because both species are seldom isolated, likely due to the capacity of *P.* ×*alni* to replace its parents under most environmental conditions (22, 36).

Comparing the genetic characteristics of *P. uniformis* isolates to those of the *P. uniformis* subgenome highlighted interesting information. All diversity indexes used in this study, including estimations within watershed/country populations, were extremely low for the European *P. uniformis* isolates and for the reconstructed *P. uniformis* subgenome. These results differ from diversity indexes estimated for North American isolates, which were consistently higher. This indicates that *P. uniformis* is present in Europe as very few genotypes dispersed throughout the continent. Despite this low diversity, *P. uniformis* seems to have presented a strong geographic differentiation in Europe, as shown by MSN, PCA, and by *F*st and *R*st, estimated for the *P. uniformis* subgenome. However, it appears that there are genotypes other than those described for *P. uniformis* by Aguayo et al. (19). In fact, some *P.* ×*alni* isolates exhibited *P. uniformis* subgenomes that were not attributable to any *P. uniformis* isolate sampled so far. A hypothesis would be that they were not recovered because of too-low sampling pressure. It has, for example, been suggested that *P. uniformis* is well adapted to cold climates, which explains its frequent isolation from Swedish rivers (20), as under these environmental conditions *P.* ×*alni* may not be able to develop and replace *P. uniformis*. This is consistent with the widespread presence of *P. uniformis* in cold regions, such as Alaska and Oregon (18, 37). *P. uniformis* from colder locations could hide new diversity, and other genotypes may be discovered. The characterization and comparison of new *P. uniformis* individuals from these locations could bring new insights into the origins of both *P. uniformis* and the hybrid *P.* ×*alni*.

The genetic characteristics of *P.* ×*multiformis* and of the *P.* ×*multiformis* subgenome were also compared. *P.* ×*multiformis* is an extremely rare species, and only a few European strains have been sampled so far. However, after intense sampling in France and Belgium and the contribution of European colleagues, we were able to gather a *P.* ×*multiformis* collection of 39 isolates. Overall, *P.* ×*multiformis* exhibited low polymorphism. However, diversity indexes within the species showed that there is some diversity, and 5 *P.* ×*multiformis* MLGs were observed. Concerning the *P.* ×*multiformis* subgenome, the number of *P.* ×*multiformis* subgenome MLGs increased to 13. However, diversity indexes computed for the *P.* ×*multiformis* subgenome were smaller than for *P.* ×*multiformis*. When genotypic richness (estimated by eMLG rarefying to 39 samples) was computed for the *P.* ×*multiformis* subgenome, it was found to be similar to that of *P.* ×*multiformis*, suggesting that some diversity within *P.* ×*multiformis* effectively exists but has not been sampled. It must be considered that *P.* ×*multiformis* has been described as a polyploid product of an interspecific hybridization between two unknown *Phytophthora* species (15, 17) and that it may present diversity levels close to those of *P.* ×*alni*. Sampling complications, added to the low polymorphism exhibited by the microsatellite markers, make the characterization of *P.* ×*multiformis* a difficult task. *P.* ×*multiformis* diversity is far from being well understood, and if possible, more samples should be included to make more-robust genetic inferences and determine its putative origin. We can, however, suggest that this sampling effort should be performed in areas were *P.* ×*alni* is not present, including samples from other European alder species and integrating asymptomatic or nondeclining trees, as was the case for the Durance and Marais-Poitevin populations.

Our study allowed us to determine some genetic characteristics of the hybrid *P.* ×*alni*. Simulated sexual crosses between the two parental species allowed us to identify *P. uniformis* and *P.* ×*multiformis* putative progenitors for 89% of the *P.* ×*alni* isolates and to assess its diversity, revealing at least 30 different MLGs. As both U and M mtDNA patterns could be found in isolates exhibiting the same microsatellite MLG, these are not likely to be true clonal lineages but rather products of several hybridization events involving the same parental MLGs. Moreover, this constitutes supplementary pieces of evidence that the sexual hybridization took place in both directions, with both parents potentially acting as the antheridial strain, as previously shown by Ioos et al. (15). In this regard, the predominance of the M over the U mtDNA in *P.* ×*alni* is also consistent with the pattern found by Ioos et al. (15). Despite the fact that during sexual hybridization both parents could act as the antheridial strain, it seems that one is often favored, leading to an unequal transmission of the mtDNA (38, 39). Bringing together this evidence, we estimated that at least 13 different hybridization events occurred during the genesis of *P.* ×*alni*. These multiple hybridization events have shaped the geographical structure of *P.* ×*alni* populations. Although no obvious geographical pattern could be demonstrated for the mtDNA and for the *P.* ×*multiformis* subgenome, a clear spatial pattern was shown for the *P. uniformis* subgenome, indicating that *P. uniformis*, described as an invasive species (19), had a major role in shaping this structure. At least two clusters differentiated by their putative *P. uniformis* parents exist within *P.* ×*alni*. While cluster A, with *Pu*-E1 and *Pu*-E3 putative progenitors, is found throughout Europe, cluster B, with the *Pu*-E2 MLG progenitor, is preferentially found in Eastern Europe. Although *P*×*a*-1 MLG, which belongs to cluster A, became dominant in Europe, MLGs deriving from the *Pu*-E2 subgenome were not replaced by this MLG in Eastern Europe. This suggests that the different hybridization events that occurred in several European areas shaped the *P.* ×*alni* population structure and that more than one single hybridization center occurred, from which the different MLGs arose and then dispersed. This pattern is not unexpected for an infertile hybrid like *P.* ×*alni* (40) and can be explained by restricted gene flow between populations, founder effects resulting from the introduction of a limited number of parental genotypes, absence of recombination, and spread of few genotypes within its populations.

Overall, diversity within *P.* ×*alni* was low. In particular, a

dominant microsatellite MLG, including more than 80% of the isolates (including both U and M types), was observed. This largely explains the low clonal richness estimated for *P.* ×*alni*. Although genetic differentiation over the years was not evident by *F*st or *R*st estimations, we observed a declining tendency of clonal richness with an increased frequency of this dominant MLG over time. This could be explained by genetic drift, which can eliminate rare genotypes from local populations (41), especially if the populations experience bottlenecks. These can be associated with human intervention such as eradication: however, management of alders in Europe is limited (42). Adverse climatic events that negatively affect the pathogen population may better explain the occurrence of bottlenecks. Indeed, population crashes after an unfavorable season offer an advantage to already well established genotypes during the recolonization process and may thus cause the extinction of rare genotypes (43). This applies to *P.* ×*alni*, as the pathogen has been shown to be highly susceptible to both high summer and low winter temperatures (44, 45). Another explanation for the decrease in clonal richness could be selection, which results in shifts in the frequency of genotypes over time when fitness differences are large (46). This may result from differences in adaptation to their host and/or environment. For example, Chandelier et al. (47) found that, while little variability in aggressiveness toward *A. glutinosa* exists within *P.* ×*alni*, the sporulation capacity is isolate dependent. However, whether genetic drift or selection is the mechanism that enables one MLG to finally predominate is currently not clear. Resolving this question would require an analysis of life history traits determining the fitness of *P.* ×*alni* (48). Nevertheless, our results illustrate how the diversity of an emerging oomycete may decrease during or shortly after the invasion process.

Finally, this study illustrates the benefits of studying the parental subgenomes present in an interspecific hybrid when the parental species are difficult to sample, rare, or even extinct. These subgenomes represent a picture of the "fossilized" diversity of the parental species.

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