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Genetic structure of *Aphanomyces euteiches* populations sampled from United States and France pea nurseries

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Abstract *Aphanomyces euteiches* Drechsler is an oomycete pathogen of leguminous crops that causes root rot, a severe disease of pea (*Pisum sativum* L.) worldwide. An improved understanding of the genetic structure of *A. euteiches* populations would increase knowledge of pathogen evolution and assist in the design of strategies to develop pea cultivars and germplasm with stable disease resistance. Twenty six primers pairs were used to amplify Sequence Related Amplified Polymorphisms (SRAP) among 49 *A. euteiches* isolates sampled from pea. A total of 190 polymorphic SRAP bands were generated, of which 82 were polymorphic between all the *A. euteiches* isolates. The percentage of polymorphic bands per primer pair ranged from 22 to 75%. According to the PIC value estimated for each marker, 60% of the SRAP markers were highly to reasonably informative (PIC > 0.25). Genetic structure of *A. euteiches* populations sampled in different American and French locations showed low to high genetic diversity within populations. The largest variation occurred within countries, with a total estimated genetic diversity of 0.477 and 0.172 for American and French populations, respectively. This was particularly evident from a principal component analysis (PCA) and a Minimum Spanning Networks (MSN) based on genetic profiles of isolates, which generated two different clusters, one corresponding to the French isolates and four American isolates (MV1, MV5, MV7, Ath3), and the other to American isolates. *A. euteiches* populations from cultivated pea in France appeared as a single unstructured population, whereas American isolates of *A. euteiches* diverged into three different populations.

Keywords Oomycota · SRAP · Genetic diversity · Root rot

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Introduction

Aphanomyces euteiches Drechsler is an oomycete pathogen of legumes, which causes the devastating root rot of pea (*Pisum sativum* L.) worldwide (Kraft and Pfleger 2001). In Europe, *A.euteiches* was first observed in Norway in 1925 (Sundheim and Wiggen 1972), and was

reported a few years later in France (Labrousse 1933), where it has been considered the most important pathogen of pea since 1993 (Didelot and Chaillet 1995). Initially considered to be only a pathogen of pea (Scott 1961), *A.euteiches* was later reported to also infect other legume species including common bean, broad bean, faba bean, clover, and alfalfa (Pfender and Hagedorn 1982; Greenhalgh and Merriman 1985; Lamari and Bernier 1985; Burnett et al. 1994; Tivoli et al. 2006; Moussart et al. 2008).

The most promising methods for controlling this disease are crop rotations and the use of cultivars with partial resistance. Knowledge of the genetic diversity and structure of *A. euteiches* populations contributes to the development of disease control strategies and selection of lines with efficient resistance against a variability of isolates. Several questions concerning the evolutionary processes occurring in *A. euteiches* populations remain unanswered. Study of plant pathogen diversity principally aims to identify which evolutionary forces are or will have more influence on the evolution of pathogen populations and predict their ability to evolve (Milgroom and Peever 2003; De Meeus et al. 2007). In general, pathogens respond to the selection imposed by their hosts through the development of new virulent strains. Many populations of fungal plant pathogens undergo bottleneck effects or founder events (Milgroom et al. 2008; Travadon et al. 2011). Both have a similar effect by reducing the amount of genetic diversity in a population. Other pathogen populations present a genetic expansion possibly due to an increase in host availability (Travadon et al. 2011). Thus, if we want to maintain sustainable pea crop production in an agricultural system, and maintain the partial resistance efficiency of pea cultivars, it is necessary to understand how genetic diversity is maintained or developed within *A. euteiches* populations, and whether cultural practices will impact diversity. For instance, incorporating knowledge of a pathogen's population structure into breeding for disease resistance may provide insight into the potential long-term and global effectiveness of resistant breeding lines.

Prior studies have used random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers to examine genetic diversity in populations of *A. euteiches* (Malvick et al. 1998; Malvick and Percich 1998; Mueller and Wolfenbarger 1999; Grünwald and Hoheisel 2006). They detected different genetic subpopulations in North America, which differed by host of origin and host preference (Malvick et al. 1998; Grünwald and Hoheisel 2006). All populations of *A. euteiches* that infected pea showed significant linkage disequilibrium between markers, which supported an important role for selfing in shaping the genetic structure of these populations (Grünwald and Hoheisel 2006).

The degree and spatial distribution of genotypic diversity within *A. euteiches* populations gave rise to contradictory reports. Malvick and Percich (1998) found high genotypic diversity within fields using RAPD markers, but no genotypic differentiation between populations from different field locations in Wisconsin, Minnesota and Oregon. In a subsequent study based on AFLP markers, Malvick et al. (2009) detected high levels of genotypic diversity at all scales but with no geographical structure in alfalfa-infecting populations in Illinois. However, by using similar AFLP markers, Grünwald and Hoheisel (2006) also found high genotypic diversity within field samples in Oregon and Washington State, but observed an even higher genetic diversity at the regional scale and a significant genotypic differentiation between fields. In Sweden, two *A. euteiches* host-specific groups ("pea" and "vetch") could be differentiated by isozyme analysis, but no geographic structure of the pathogen populations was observed (Levenfors et al. 2003; Rosendahl 2007). In France, isolates of *A. euteiches* were characterized based on pathogenicity to legume species and pea lines (Wicker et al. 2001; Wicker and Rouxel 2001; Moussart et al. 2008), leading to the definition of virulence phenotypes on legumes and pathotypes on pea. All the isolates from France belonged to one major pathotype (pathotype I) and a wide range of aggressiveness was observed among these isolates. Isolates from pathotype III were identified only in the United States. However, these phenotypic groups were not genotypically clustered (Wicker 2001). Inconsistent conclusions between studies regarding the genetic structures of *A.euteiches* populations may be due in part to dissimilarities in the type of markers used to detect genetic variation. Sequence-related amplified polymorphism (SRAP) (Li and Quiros 2001) PCR, is simple dominant marker technique, and effective for producing genome-wide fragments with high reproducibility and versatility (Robarts and Wolfe 2014). These markers were originally developed for gene tagging in *Brassica oleracea* L. to specifically amplify coding regions of the genome. The primers are 17 or 18 nucleotides long and consist of core sequences, which are 13 to 14 bases long. The first

10 or 11 bases starting at the 5′ end are "filler" sequences, maintaining no specific constitution. These are followed by the sequence CCGG- (forward) or -AATT (reverse). This core is followed by three selective nucleotides (random) at the 3′ end (Li and Quiros 2001). SRAPs have also been used successfully to evaluate diversity in nematodes (Li et al. 2009), oomycetes (Chen et al. 2009) and several species of fungi (Sun et al. 2006; Yu et al. 2008; Pasquali et al. 2010; Robarts and Wolfe 2014). These markers have proven to be robust and highly variable, and are attained through a significantly less technically demanding process. SRAP markers have been used primarily for agronomic and horticultural purposes, developing quantitative trait loci in advanced hybrids and assessing genetic diversity of large germplasm or pathogen collections. A recent review, based on 171 comparative works using SRAP markers underlined that these markers would be a useful and powerful molecular tool in a diversity of fields by providing an easy-to-use variable marker.

The work reported here had two main aims: first, to develop and test the efficacy of SRAP markers to detect genetic polymorphisms among *A. euteiches* isolates, and to characterize the genetic structure within and among *A. euteiches* populations occurring in US and French field disease nurseries.

Materials and methods

Sampling of French and US isolates of A. euteiches Forty-eight isolates of *A. euteiches* from American nurseries and French nurseries were used in this study (Table 1). Soil samples were collected in October and November 2005 from an international *Aphanomyces* field network consisting of four American nurseries (Le Sueur, Minnesota 44°27′N, 93°54′W; Athena, Oregon 45°48′N, 118°29′W; Mount Vernon, Washington 48°25′N, 122°2′W; Pullman, Washington 46°43′N, 117°9′W) and three French nurseries (Dijon, Côte d'Or 47°3′N, 4°1′E; Templeux-le-Guérard, Somme 49°57′N, 3°8′E; Riec-sur-Belon, Finistère 47°52′N, 3°42′W). Six of these nurseries, naturally infested with *A. euteiches*, were described in Pilet-Nayel et al. (2005) and Hamon et al. (2011, 2013). The seventh nursery, Mount Vernon, was described in Grünwald and Hoheisel (2006). Ten soil samples (numbered 1–10) were collected in each nursery in a "W" design at a 20-25 cm depth and stored at 5 °C.

Table 1 *A. euteiches* isolates obtained from fields in France (3 locations) and the United States (4 locations)

Country	Location	Number of Isolates	A. euteiches isolates
France	Riec Sur Belon (Finistère)	5	Ri2
			Ri4
			Ri7
			Ri8
			Ri10
			RB84
	Templeux (Somme)	10	Tpx1
			Tpx2
			Tpx3
			Tpx4
			Tpx5
			Tpx6
			Tpx7
			Tpx8
			Tpx9
			Tpx10
	Dijon (Côte d'Or)	9	Di1
			Di2
			Di3
			Di4
			Di5
			Di6
			Di7
			Di8
			Di10
US	Athena (OR)	7	Ath1
			Ath2
			Ath3
			Ath4
			Ath5
			Ath6
			Ath7
	Le Sueur (MN)	8	LS1
			LS2
			LS3
			LS5
			LS6
			LS7
			LS8
			LS10
	Pullman (WA)	4	Plm1
			Plm2
			Plm3
			Plm4
	Mount Vernon (WA)	5	MV1
			MV3
			MV4
			MV5
			MV7

A. euteiches was baited from each soil sample using the pea susceptible genotype cv Baccara, as described by Moussart et al. (2008), and one isolate per soil sample from which disease symptoms were observed was randomly selected. A collection of 48 isolates was thus constructed, including 24 isolates from the French nurseries, i.e. nine from Dijon (isolates Di1 to Di8, and Di10), 10 from Templeux-le-Guérard (Tpx1 to Tpx10) and five from Riec-sur-Belon (Ri2, Ri4, Ri7, Ri8 and Ri10) and 24 isolates from the American nurseries, i.e. seven from Athena (Ath1 to Ath7), eight from Le Sueur (LS1 to LS3, LS5 to LS8, and LS10), five from Mount Vernon (MV1, MV3 to MV5, and MV7) and four from Pullman (Plm1 to Plm4). All 48 isolates were single spored, grown and maintained on Corn Meal Agar at 10 °C as described by Malvick et al. (1998). Three singles spores isolates were used as controls; Ae109, Wicker and Rouxel 2001; (RB84, Moussart et al. 2007; and MF1, Malvick and Grau 2001).

Culture conditions, DNA extractions and quantification A. euteiches mycelial explants were grown for six days at 25 °C on CMA. Seven to ten agar discs (3 mm diameter) per culture were then transferred to peptone-glucose-rifampicin broth and grown for six days at 25 °C. Mycelial mats were then vacuum-filtered on Whatman paper, rinsed three times with sterile water, and transferred to Eppendorf tubes. The DNA extraction protocol from Goodwin and Lee (1993) was used with the modifications described by Wicker (2001). Lyophilized (24 h) mycelia were ground (30 s, speed 4.5) in a Bio 101 blender (Fastprep products, Q-Bio Gene) without any buffer. Ground mycelia was then mixed with lysis buffer (about 30 mg mycelial powders per 500 μL lysis buffer) and ground a second time as described above. DNA was resuspended in TE0.1 buffer (Tris-HCl 1 M, pH 8,0; EDTA 0.5 M, pH 8,0; distilled water) and stored at −20 °C. DNA samples were quantified using a Nanodrop 1000 spectrophotometer (Thermo FisherScientific) and diluted to 10 ng/μl.

SRAP primers and reactions SRAP amplification was first performed on the three *A. euteiches* reference isolates (Ae109, Wicker and Rouxel 2001; RB84, Moussart et al. 2007; and MF1, Malvick and Grau 2001) with 270 primer combinations (combinations of 18 forward primers and 15 reverse primers), using Faststart Taq (Roche, Lewes, UK) in a Geneamp 9700 PCR system (ABI, Warrington, UK). SRAP were amplified according to the protocol described by Li and Quiros (2001) with some modifications. Briefly, 10 ng of DNA (1 μl) was amplified in a 20 μl reaction mix containing 1 unit of Taq (5 Unit/μl; GoTaq, Promega), 1× PCR buffer (GoTaqFlexi, Promega), 37.5 ng of each primer, 200 μM of each dNTP, and 1.5 mM $MgCl_2$. PCR was performed using the following thermocycling profile: 10 min at 95 °C followed by five cycles of 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 1 min, then 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. Amplicons were electrophoresed in 1.5% agarose gels using a 123 bp ladder (Life Technologies) as a size marker. The gels were stained with Sybr Gold and bands were visualized with a DNA analyser Gene ReadIR 4200 (LI-COR Inc., Lincoln, NE, USA). Gels were scored for all polymorphic amplicons. It was assumed that amplicons of the same molecular weight in different individuals were identical. Amplicon presence was indicated by a (1) and absence by a (0). SRAP reactions were performed independently three times, using the same set of primers with reference isolates (RB84, Ae 109) and a random sample of 10 isolates from the collection, and independent DNA preparations of the same isolates to estimate the repeatability of fragment scoring. The allelic diversity or polymorphism information content (PIC) was measured for each selected polymorphic SRAP as described by Botstein et al. (1980) for the 48 *A. euteiches* strains.

Data analysis The genetic structure of the different *A. euteiches* populations studied was analysed according to their geographic origin and location.

A multilocus genotype was constructed for each isolate by combining data from single SRAP alleles. Each SRAP fragment size was considered a locus with two alleles (fragment presence or absence). Data were used to define multilocus genotypes (MLGs) and check for repeated MLGs, i.e. the strains sharing the same alleles at all loci, using the Microsoft EXCEL add-in GenAlEx version 6.5 (Peakall and Smouse 2012). The number of different alleles (Na), Shannon's information index (I) and the expected heterozygosity (*He*) were computed using GenAlEx for each population and sub-population. Multilocus genotypes (MLGs) and the number of isolates sharing the same MLG at each site were identified by Arlequin (Excoffier et al. 2005).

To measure the genotypic diversity, genotypic evenness was evaluated using the index R = (G-1)/(N-1) (Grünwald et al. 2003).

Partition of molecular diversity across and within countries, as well as across and within locations, was studied using analysis of molecular variance (AMOVA) (Excoffier et al. 1992; Lynch and Milligan 1994). AMOVA was performed using Arlequin 3.1 (http://cmpg.unibe.ch/software/arlequin3) (Excoffier et al. 2005). The significance was estimated from 1000 randomizations calculated with the software multiLocus 1.2.2.

Linkage disequilibrium in the *A.euteiches* dataset was analyzed using the software multiLocus 1.2.2 to estimate the index of association, *Ia* (Burt et al. 1996). The association between the scored alleles was estimated by comparing variance of the genetic distances in the current dataset to the mean variance of 1000 re-sampled datasets. An *Ia* value differing significantly from 0 rejects the null hypothesis of random mating, whereas panmixis and sexual recombination is expected to result in a *IA* value close to zero (Burt et al. 1996).

Principal component analysis (PCA) was performed using the procedure available in the package adegenet (Jombart 2008) of the statistical freeware R version 2.7.2 (The R foundation for Statistical Computing 2008). PCA has an important advantage over methods such as the Bayesian clustering algorithm implemented in Structure 2.2 (Pritchard et al. 2000; Falush et al. 2003) as it does not require strong assumptions about an underlying genetic model, such as the Hardy–Weinberg equilibrium or the absence of linkage disequilibrium between loci (Jombart et al. 2009).

Minimum spanning networks (MSN) are a great way to visualize relationships among individuals. Particularly for clonal organisms it can be a more powerful visualization tool than trees (Kamvar et al. 2015). By using the package poppr from R statistical software, version 3.1.2 (©2014, The R Foundation for Statistical Computing), MSN was calculated using Nei's distance for US and French *A. euteiches* populations. Multilocus genotypes (MLG) were collapsed to multilocus haplotypes which are represented by circles containing the number of associated isolates and sized in proportion to haplotype frequency. Haplotype information could lead to more powerful tests of genetic association than single-locus analyses but it is not easy to estimate haplotype frequencies from genotype data due to phase ambiguity. The challenge is compounded when individuals are pooled together to save costs or to increase sample size. By collapsing the total allele frequencies of each pool suitably, the maximum likelihood estimates of haplotype frequencies based on the collapsed data can be calculated very quickly regardless of pool size and haplotype length (Kuk et al. 2013).

Results

SRAP markers polymorphism evaluation.

Out of the 270 selected primer combinations tested with the three *A. euteiches* isolates (Ae109, RB84, and MF1) used in the initial screening, only 20 primer combinations showed reproducible polymorphism (Table 2). Out of the 190 bands generated by these 20 primer combinations, 82 bands (43%) revealed differences between the different *A. euteiches* isolates used in this study. The percentage of polymorphic bands per primer combination ranged from 22 to 75%. The PIC value of each marker varied from 0 to 0.840 (average 0.355) for the 48 isolates studied (Table 2). According to the criteria proposed by Botstein et al. (1980), nine (45%) of the SRAP primer pairs were highly informative (PIC >0.5), three (15%) were reasonably informative (0.25 < PIC <0.5), and eight (40%) slightly informative (PIC <0.25).

Partition of population genetic diversity within and across locations and countries

The genetic structure of the four American and three French *A.euteiches* populations was investigated using the 82 polymorphic SRAP markers. Results revealed a low to high genetic and genotypic diversity, depending on the location (Table 3). Most isolates belonged to different genotypes, with multilocus analysis detecting a total of 31 different multilocus genotypes of *A. euteiches* (MLG) among the 48 isolates tested with SRAP markers. Genotypic evenness (R) ranged from 0.5 (Athena and Dijon) to 1.0 (Pullman, Mount Vernon, and Riec).

Expected heterozygosity (*He*) and gene diversity (I) values showed that the highest genetic diversity was for Mount Vernon (He = 0.502, *I* = 0.505) and the least for Dijon (He = 0.037, *I* = 0.073) populations. The largest I value occurred within the US populations, with a total estimated genotypic diversity of 0.477 vs. 0.172 for French populations of *A. euteiches* (Table 3). *He* value ranged from 0.052 (Pullman) to 0.502 (Mount Vernon) for the locations in the US, and from 0.037 (Dijon) to

Table 2 Details of primer pairs, No. of detected bands (No bands), No. and percentage of polymorphic bands, and PIC value of different SRAP markers used to evaluate genetic diversity within *A. euteiches* isolates

Primer combinations	Forward primer	Reverse primer	No bands	No of polymorphic bands	% polymorphic bands	PIC
1	F9: 5′-GTAGCACAAGCCGGACC-3’	Em2: 5′-GACTGCGTACGAATTTGC-3’	12	6	50	0.136
2	F9: 5′-GTAGCACAAGCCGGACC-3’	R7: 5′-GACACCGTACGAATTTGC-3’	14	6	43	0.166
3	F9: 5′-GTAGCACAAGCCGGACC-3’	R9: 5′-GACACCGTACGAATTTGA-3’	12	4	33	-
4	Me4: 5′-TGAGTCCAAACCGGACC-3’	R10: 5′-GACACCGTACGAATTAAC-3’	9	2	22	0.532
5	Me2: 5′-TGAGTCCAAACCGGAGC-3’	Em6: 5′-GACTGCGTACGAATTGCA-3’	10	5	50	0.480
6	F9: 5′-GTAGCACAAGCCGGACC-3’	R8: 5′-GACACCGTACGAATTGAC-3’	13	5	39	0.480
7	F8: 5′-GTAGCACAAGCCGGAAT-3’	Em6: 5′-GACTGCGTACGAATTGCA-3’	11	5	45	-
8	F12: 5′-CGAATCTTAGCCGGAGC-3’	R9: 5′-GACACCGTACGAATTTGA-3	8	4	50	0.840
9	F8: 5′-GTAGCACAAGCCGGAAT-3’	R11: 5′-GACACCGTACGAATTGCA-3’	9	6	66	-
10	F13: 5′-CGAATCTTAGCCGGCAC-3’	R14: 5′-CGAACGTCCGTAATTAAC-3’	9	4	45	0.504
11	F14: 5′-CGAATCTTAGCCGGAAT-3’	Em2: 5′-GACTGCGTACGAATTTGC-3’	6	4	67	0.320
12	F14: 5′-CGAATCTTAGCCGGAAT-3’	R8: 5′-GACACCGTACGAATTGAC-3’	8	3	38	0.536
13	F8: 5′-GTAGCACAAGCCGGAAT-3’	R7: 5′-GACACCGTACGAATTTGC-3’	8	6	75	0.120
14	F8: 5′-GTAGCACAAGCCGGAAT-3’	R8: 5′-GACACCGTACGAATTGAC-3’	9	5	56	0.179
15	F10: 5′-GTAGCACAAGCCGGAAG-3’	Em3: 5′-GACTGCGTACGAATTGAC-3’	11	3	27	0.840
16	F11: 5′-CGAATCTTAGCCGGATA-3’	Em3: 5′-GACTGCGTACGAATTGAC-3’	10	4	40	-
17	F13: 5′-CGAATCTTAGCCGGCAC-3’	Em1: 5′-GACTGCGTACGAATTAAT-3’	5	2	40	0.590
18	F11: 5′-CGAATCTTAGCCGGATA-3’	Em2: 5′-GACTGCGTACGAATTTGC-3’	8	3	38	0.510
19	F9: 5′-GTAGCACAAGCCGGACC-3’	R12: 5′-CCTTGCTACGCAATTGAC-3’	9	3	33	0.504
20	F8: 5′-GTAGCACAAGCCGGAAT-3’	R10: 5′-GACACCGTACGAATTAAC-3’	9	2	22	0.824

0.088 (Riec) for the French locations. The I values ranged from 0.073 to 0.115 among the French populations, and from 0.066 to 0.505 among the US populations (Table 3). The mode of reproduction and mating system was tested by analyzing possible linkage disequilibrium in the data sets. The statistics on the Index of association (*IA*) were significantly different from 0 in French (I_A = 1.61, *P-value* < 0.002) and American (I_A = 2.60, *P-value* < 0.002) populations, indicating that the populations were clonal.

AMOVA confirmed the lack of genetic subdivision between locations and revealed that 69.97% of the total genetic variance was partitioned within countries. A relatively low proportion of genetic variability was attributable to differences between populations of a country (11.26%). The θ statistic between the two US-FR subpopulations of *A euteiches* isolates was significant ($\theta_{FR\text{-}US}$ = 0.7369***), suggesting genetic differentiation.

PCA clearly separated US and French populations of *A. euteiches* into two distinct groups (Fig.1). However, four US isolates (MV5, MV1, MV7, and Ath3) clustered with the French isolates. The first PCA axis, separating the French from the US isolates, explained 82.4% of the variation; the second axis, separating the US isolates, explained only 3.99% of the structure.This distribution also was observed from MSN analyses (Fig.2). Similar to PCA, MSN revealed two distinct groups isolates; group 1 including all the French isolates and four US isolates (MV5, MV1, MV7, and Ath3), and group 2 included all the other US isolates (Fig.2). In each group, MSN calculation showed variation in frequency of MLGs (higher frequency for MLG 24, 26, 28, and 29 in group 1, and for MLG 6, 9, 17, and 19 in group 2).

Discussion

This study developed and applied SRAP markers to detect genetic polymorphisms among *A. euteiches* isolates and examined population genetic structure among pea-infecting *A. euteiches* isolates collected from France and the US. This is the first study taking into account the country scale for comparison of *A. euteiches* populations. This is interesting and potentially valuable, as common pea germplasm or sources of resistance (Gritton 1980) are used in both countries for improving partial levels of resistance in varieties.

The analysis of SRAP marker data showed that the population structure of the *A. euteiches* isolates was dependant on country of origin. The number of polymorphic SRAP markers used in this study was similar to the number of polymorphic AFLPs (*N* = 56) detected in a study of *A. euteiches* isolates from different US locations (Grünwald and Hoheisel 2006). These results suggest that SRAP markers are useful for detecting and quantifying genetic variation in *A. euteiches* populations. Similarly to results observed for stripe rust (*Puccinia striiformis*) (Pasquali et al. 2010) and *Phytophthora sojae* (Chen et al. 2009), SRAP markers could separate *A. euteiches* isolates on the level of subpopulation with robust bootsrap support values. However, this type of markers did not appear to reveal substantially more about population genetics and/or genotypic diversity of this pathogen than did other marker types (e.g. AFLPs and RAPDs). As observed in many comparative studies, SRAP markers provide comparable levels of variation to AFLP markers, but with significantly less technical effort and cost, and similar

Table 3 Parameters of genetic diversity for populations of *A. euteiches* sampled in the different locations and countries studied. Values indicated into brackets corresponded to results from analysis conducted with only one copy of each multilocus genotype

Scale	Site	n	G	R	Nb of polymorphic loci	He	I
Country	FR	25 (14)	19	0.75	18 (18)	0.113 (0.094)	0.172 (0.191)
	US	24 (17)	19	0.78	49 (45)	0.316 (0.344)	0.477 (0.512)
Regional	Templeux	10 (6)	8	0.78	11 (11)	0.056 (0.088)	0.115 (0.127)
	Dijon	9 (6)	5	0.50	6 (6)	0.037 (0.055)	0.073 (0.077)
	Riec	6 (3)	6	1.00	11 (9)	0.088 (0.068)	0.122 (0.223)
	Pullman	4 (4)	4	1.00	5 (5)	0.052 (0.047)	0.066 (0.066)
	Mount Vernon	5 (5)	5	1.00	44 (44)	0.502 (0.344)	0.505 (0.505)
	Athena	7 (2)	4	0.50	41 (41)	0.237 (0.333)	0.380 (0.486)
	Lesueur	8 (6)	6	0.71	8 (8)	0.067 (0.068)	0.097 (0.098)

n number of isolates, *G* number of distinct multilocus genotype, *R* genotypic richness, *He* Expected heterozygosity (Nei, 1973), *I* Shanon information's index as a measure of gene diversity

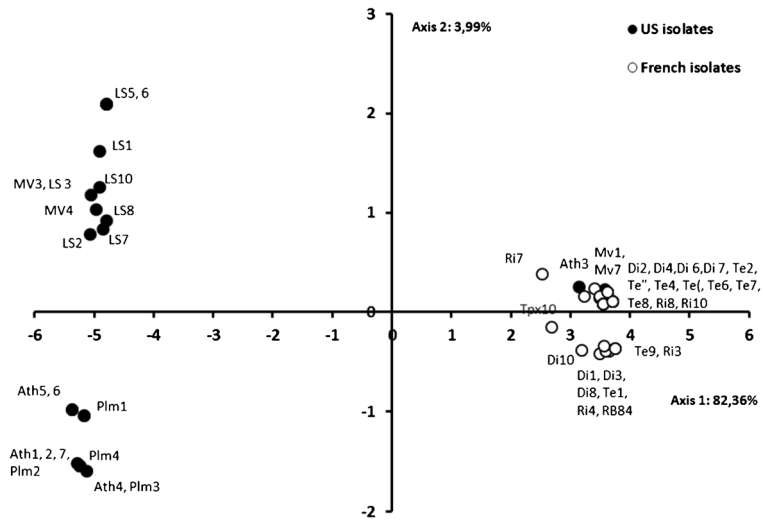

Fig. 1 Principal component analysis (PCA) based on 82 SRAP marker loci on 48 A. *euteiches* isolates sampled from different locations in the US (Ath: Athena, LS: Le Sueur, MV: Mount Vernon, Plm: Pullman) and France (Di: Dijon, Ri: Riec sur Belon, Tpx: Templeux). A. *euteiches* isolates represented according to their country of origin

levels of band-pattern variability and reproducibility (Li and Quiros 2001; Levi and Thomas 2007; Liu et al. 2007; Wang et al. 2007; Lou et al. 2010). Further, SRAPs are mainly dominant as observed in our study, but codominance has been identified in up to 20% of SRAP markers examined (Li and Quiros 2001), which is a higher rate than previously described for AFLP (Mueller and Wolfenbarger 1999). In our study, even if they revealed limited new information and polymorphisms SRAPs were helpful tools for analysing genetic diversity of *A. euteiches* populations which is poorly reported in the literature. Recently, Mieuzet et al. (2016) developed and screened a microsatellite-enriched small-insert genomic library for identification of *A. euteiches* SSR containing sequences. Fourteen out of the 48 primer pairs designed to amplify SSR, produced unambiguous polymorphic products. In the coming years, it will be possible to develop SNP markers all over the genome by mining the *A. euteiches* genomic sequence (Gaulin et al. 2007, 2008) and NGS methodologies for genome re-sequencing. SRAP markers are polymorphic but reveal a low level of diversity within populations. These new tools will be helpful to study genomic regions under selection, to identify effectors, and to identify other markers or genome region directly implicated in the adaptative and specialization processes leading *A. euteiches* to easily be adapted to different legume species.

Based on the genetic polymorphism generated by the SRAP markers, we examined the genetic structure of sub-populations of *A. euteiches* originating from different nurseries used for screening plant material for resistance in France and the US. This is the first report to examine genetic diversity between isolates of *A. euteiches* from France and the US. Results demonstrated that genetic structure of *A. euteiches* populations were highly dependant on geographic origin. A low to high genetic diversity was observed within the different locations in France and in the US, with most isolates corresponding to a single multilocus genotype (31 different MLGs within 49 isolates). At the field scale, we observed gene diversity values (I) similar to those previously observed using AFLPs (Grünwald and Hoheisel 2006). This study was based on a limited sample size per geographic location and a moderate number of geographic locations. The isolates used in the current study were baited by using a standard procedure and the same weight of soil. However, the success of baiting was dependant on the quantity of pathogen initially present in the soil, resulting in an unbalanced number of isolates collected from location. Indeed, for soil with a low inoculum potential it was generally more difficult to recover and bait a large number of isolates.

This work showed that SRAP markers are useful to highlight the genetic structure of *A. euteiches* populations in French and American nurseries. However, only a relatively low frequency of SRAP primer pairs produced polymorphisms and these useful primer pairs each detected, on average, two polymorphic amplicons. Genetic analysis of French and US populations showed a low to high genetic diversity within these populations and a separation by geographical origin possibly indicating specialization of pathogen to host genotype. Possible evolutionary scenarios for *A. euteiches*

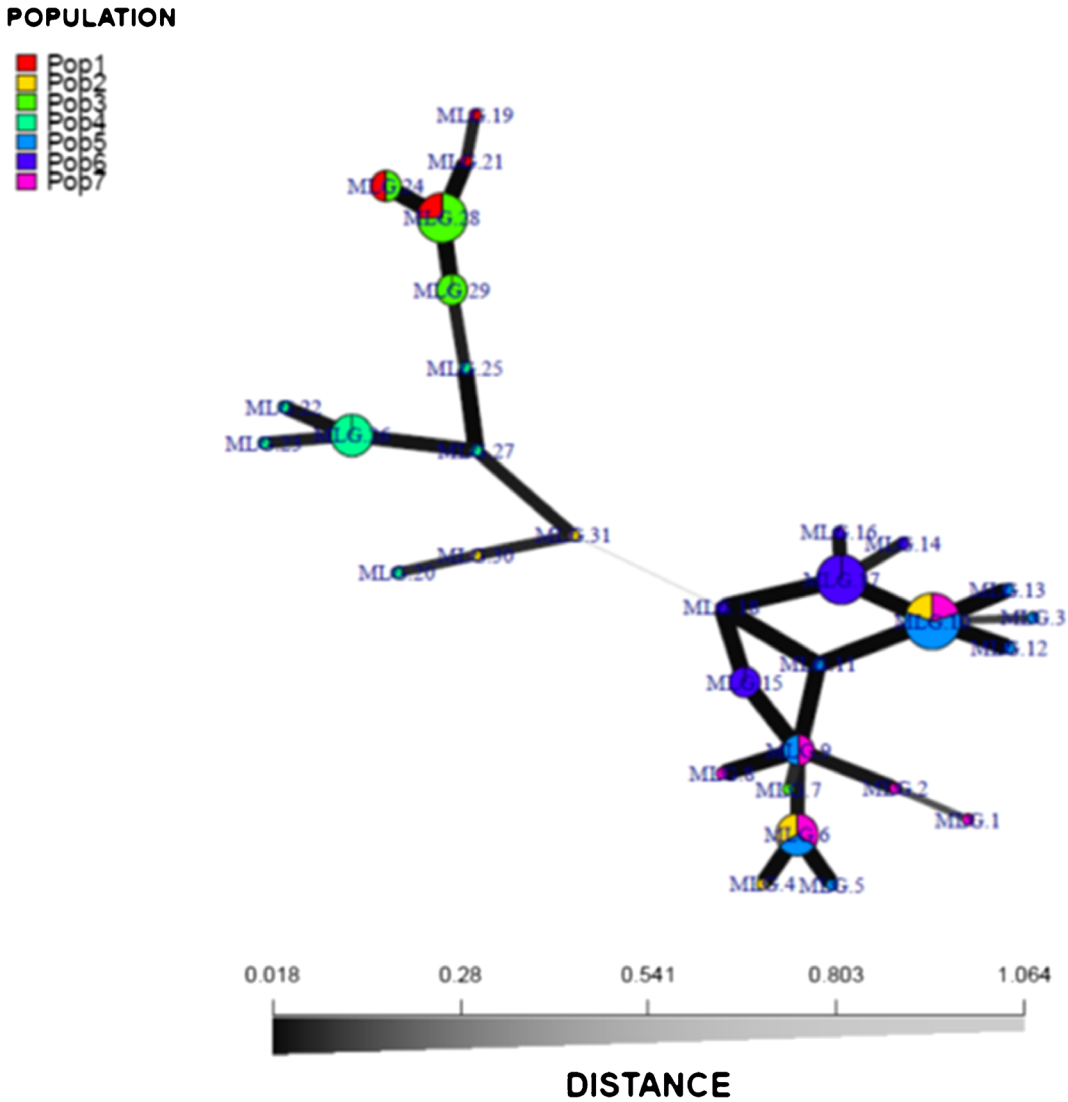

Fig. 2 Minimum spanning network (using Nei's distance) for the US and French *A. euteiches* populations. Multilocus genotypes (MLG) were collapsed to multilocus haplotypes which are represented by circles containing the number of associated isolates and sized in proportion to haplotype frequency. Pop1: Pullman (Plm), Pop2: Mount Vernon (MV), Pop3: Athena (ath), Pop4: Le Sueur (LS), Pop5: Templeux (Tpx), Pop6: Dijon (Di), Pop7: Riec sur Belon (Ri)

populations will need to be investigated using more extensive and appropriate molecular tools. Differences between the US and French Aphanomyces populations may be related to the history of legume cropping and disease development in both country. *A. euteiches* was first described by Jones and Drechsler (1925) after an extensive survey conducted in 1924 and recognized as one of the most damaging soil-borne pathogens on this crop (Jones and Linford 1925; Gaulin et al. 2007). In France *A. euteiches* has caused major losses in pea crops only since 1993 (Didelot and Chaillet 1995). Thus, American populations of pea-infecting *A. euteiches* may have started to differentiate earlier into subpopulations with difference in virulence on peas, and alfalfa (Holub et al. 1991; Malvick et al. 1998). Thus, as the selection by local host seems to be an active process, it would be essential to complete sequencing of the *A. euteiches* genome to better understand fully the pathogenicity, biology and evolutionary process of this devastating pathogen (Gaulin et al. 2007). Understanding and predicting the genetic and pathogenic structure within populations will also improve rotation management and deployment of resistance QTL.

Our data support the idea that exchanges between *A. euteiches* populations are possible, even at large distances. It is therefore essential to obtain a more comprehensive view of the mechanisms shaping genetic diversity and pathogenicity within *A.euteiches* populations. This will definitely require the conjunction of phenotypic and genetic data, and possibly also an extended sampling effort to increase the number and size of local populations investigated. It would be useful to survey other locations in the US to improve understanding of the genetic and pathogenic structure of *A. euteiches* populations in the US. Since *A. euteiches* is able to infect different legumes species (Sherwood and Hagedorn 1958), it would also be useful to obtain information regarding crop rotation histories of sampling locations, which may identify processes that can shape the genetic structure and pathotype distribution of *A. euteiches* populations.

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Compliance with ethical standards The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The research presented in this manuscript does not implicate nor Human Participants and/or Animals.

CLM and GV were the principal investigator of this research project. They conducted the research experiments and contributed to the data analysis and to drafting the manuscript. CO contributed to the *A. euteiches* strains baiting, and to the drafting of the manuscript. MLP, AB, AM and DA contributed to the drafting of the manuscript. All authors read and approved the manuscript.

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