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Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. I – Is specific seedling resistance selected?

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Abstract Dynamic management has been proposed as a complementary strategy to gene banks for the conservation of genetic resources. The evolution of frequencies of genes for specific resistance towards powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*) in populations of a French network for dynamic management of bread wheat genetic resources was investigated after 10 years of multiplication without human selection. The objective was to determine whether specific resistance gene diversity was maintained in the populations and whether any changes could be attributed to selection due to pathogen pressure. Seven populations, originating from four of the network sites, were characterized and compared to the initial population for six specific resistance gene frequencies detected by nine *Blumeria graminis* f. sp. *tritici* isolates. Diversity decreased at the population level, but because of a strong differentiation between the populations, this diversity was maintained at the network level. The comparison of *Fst* parameters estimated on neutral markers (RFLP) and on resistance gene data revealed that in two of the populations specific resistance genes had been selected by pathogen pressure, whereas evolution in two other populations seemed to be the result of genetic drift. For the three last populations, conclusions were less clear, as one had probably experienced a strong bottleneck and the other two presented intermediate *Fst* values. A dynamic management network with sites contrasted for pathogen pressure, allowing genetic drift in some populations and selection in others, appeared, at least on the short term, to be a good tool for maintaining the diversity of genes for specific resistance to powdery mildew.

Key words Composite populations · *Triticum aestivum* · *Blumeria (Erysiphe) graminis* f. sp. *tritici* · Selection · Drift

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Introduction

The conservation of genetic resources has become a major objective. Different methods of conservation have been developed during the last decades. Static conservation in gene banks is the most frequently used method world-wide. The objectives of gene banks are to conserve a fixed image of the genetic diversity present at the time of the collection and to avoid as much as possible any evolution of this variability. Hence, as the conserved resources are isolated from the environment, which is continuously changing (evolution of the pathogen populations, global change...), they might become obsolete over a short period of time (according to the "Red Queen hypothesis", Van Valen 1973). Dynamic management is a complementary strategy to gene banks. It aims to maintain evolutionary processes on broadly based populations grown generation after generation in a network of different sites contrasted for pedo-climatic and biotic conditions. Studying the evolution of pathogen resistance in such a system is one way to assess the effects of biotic pressures on composite populations and to test their ability to adapt to rapid changes.

In barley (*Hordeum vulgare* L.) composite cross populations grown in Davis, California, the frequencies of resistance alleles towards *Rhynchosporium secalis* (Oud) Davis increased (Allard 1988, 1990). This increase was correlated to a higher resistance of the barley populations towards the pathogen population (Jackson *et al.*

1978, 1982; Saghai-Maroof *et al.* 1983; Webster *et al.* 1986). In the case of powdery mildew [caused by *Blumeria graminis* (DC.) Golovin ex Speer (syn. *Erysiphe graminis* DC.*)* f. sp. *hordei* Em. Marchal] resistance, in the same populations grown in California or others in Montana, two areas where powdery mildew is not a major disease, no relationship could be established between pathogen pressure and the evolution of specific resistances (Saghai-Maroof *et al.* 1983; De Smet *et al.* 1985). In 1974, large samples of seeds from generations 10, 20 and 30 of composite cross V, created in 1937 (Harlan *et al.* 1940), were transferred to Cambridge, UK, where powdery mildew was much more prevalent, and cultivated there for 10 years. The frequency of plants resistant to the open-air powdery mildew spores increased significantly (Ibrahim and Barrett 1991). These results indicated that when pathogen pressure is high enough, it can act as a selective pressure and influence the evolution of specific resistance in composite populations.

In 1984, an experiment involving the dynamic management (DM) of genetic resources of bread wheat (*Triticum aestivum* L.) was initiated in France (Henry *et al.* 1991). Segregating populations derived from the crossing of varieties and lines of various origins (two predominantly selfing populations and one outcrossing due to a male sterility gene) were used as the initial gene pools. Seeds samples from these pools were distributed throughout a French network. Since then, each population has been cultivated in the same site, under the same agricultural conditions (extensive or intensive), without any conscious selection, and isolated from each other and from any other wheat plot to avoid pollen exchange.

The study of three wheat DM populations derived from each of the three initial pools after 8 years of cultivation under extensive farming conditions showed that all of the identified genes for specific resistance to powdery mildew [caused by *Blumeria graminis* (DC.) Golovin ex Speer (syn. *Erysiphe graminis* DC.*)* f. sp. *tritici* Em. Marchal] present in the initial pools were maintained (Le Boul'ch *et al.* 1994). The evolution of the frequencies of these genes depended mainly upon the initial genetic context (initial pool), with an effect of the multiplication site. However, because the specific resistance genes that had increased the most were overcome at the time of the study, it was not possible to conclude whether any changes were the result of selection due to pathogen pressures or not. Answering this question was one of the major objectives of the study presented here. For this we investigated a large sample of populations originating from one of the selfing genetic pools, called PA. The experiment was conducted on lines derived from these populations. This allowed us to characterize each line for both specific resistance at the seedling stage and adult-plant resistance (the results concerning adult-plant resistance are presented in a second paper). These lines had been selected for their short stature in order to investigate the variability of direct interest in breeding programmes.

In this paper we present our results on the evolution of specific resistance genes towards powdery mildew in DM populations after 10 years of cultivation at four locations subjected to various degrees of powdery mildew pressures. We investigated whether the evolution of specific resistance gene frequencies could have been due to selection and whether this selection could have resulted from pathogen pressure. For this, a measure of the between-population differentiation (Wright *Fst* parameters) was estimated on powdery mildew-specific resistance gene data, and this measure compared to *Fst* parameters previously estimated on restriction fragment length polymorphism (RFLP) markers (Enjalbert *et al.* 1999a). As RFLP loci are supposed to be neutral with respect to selection, they were used as a reference for evolution under genetic drift, even though some of them were certainly subjected to hitch-hiking effects.

Materials and methods

Origin of the DM populations

The initial pool (PA0) was obtained from a pyramidal cross involving 16 parents representing a wide genetic basis (Enjalbert *et al.* 1999b). Sixty-nine single-seed descent lines derived from the initial PA0 pool and samples of 15–21 short inbred lines derived from seven populations of the network were characterized for their composition with respect to powdery mildew specific resistance genes. The seven populations had been cultivated for 10 years (8 in the case of Rennes) at four locations of the network (Fig. 1): Châlons-sur-Marne (CHA), Rennes (LRE and LRI), Toulouse (TOE and TOI) and Vervins (VVE and VVI). I and E stand for intensive and extensive farming methods, respectively. The intensive farming method corresponds to the classical method applied for wheat growing in the area, whereas in extensive farming method no fungicide treatment and only one-third of the N fertilizers normally used in the intensive method were applied. In Châlons-sur-Marne, only one population was available, of which the I or E status has not been taken into account because of changes in its management during the first 10 years.

Determination of the specific resistance genes

Nine powdery mildew single-spore isolates originating from France were selected for their virulence spectra (Table 1) and ability to reveal resistance genes in the parents of the PA pool and in PA populations (Le Boulc'h *et al.* 1994). We studied six resistance genes: *Pm2, Pm4b, Pm5, Pm6, Pm8* and *Mlar,* and a resistance factor recently identified in one of the parents that will be referred to subsequently as *Pmx*. *Mlar* is present in three of the parents and *Pm4b* in two of them (Table 2). The isolate collection allowed the identification of the combinations of most of these specific resistance genes.

The *Pm4a* and *Pm4b* alleles could be distinguished only by isolate 95.42 (Table 1). Most of the results concerning the *Pm4* gene were in favour of the *Pm4b* hypothesis as expected from the genotype of the parents. Nevertheless, sometimes the results did not allow us to decide whether *Pm4a* or *Pm4b* was the best hypothesis. As this situation was rather infrequent, the results concerning these alleles will be referred to as *Pm4b* in the following. The results concerning *Pm5*, a gene identified in two of the parents, will not be presented here because the isolate collection did not allow us to separate lines bearing *Pm4b* and *Pm5* from those with *Pm4b* alone. As *Pm4b* was a frequent gene in most of the populations (see results), *Pm5* frequency could not be estimated with enough precision. Furthermore, *Pm5* is only fully expressed

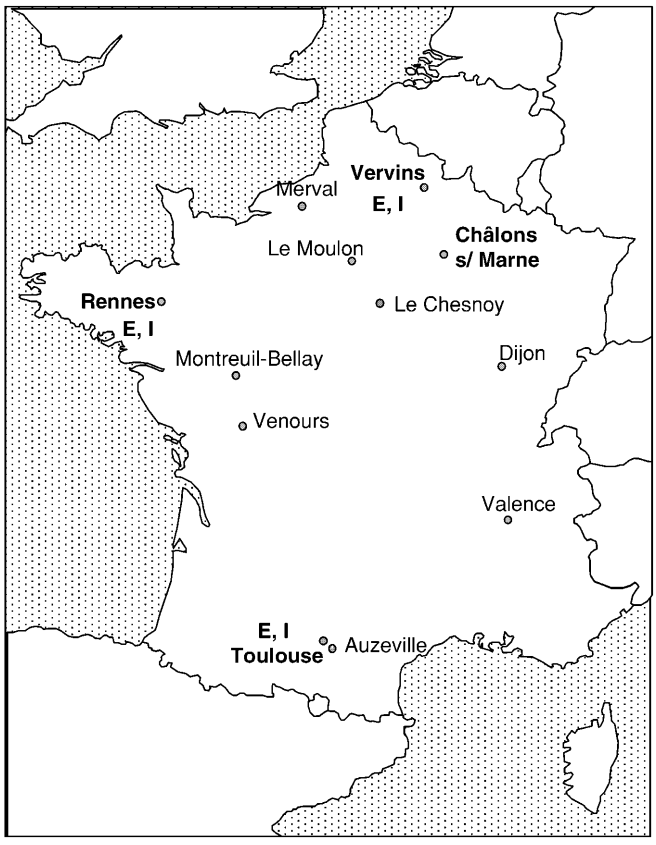

Fig. 1 Network for the dynamic management of the wheat genetic resources programme (DGER of French Agriculture Ministry, INRA, INA-PG and BRG). The seven populations studied for their powdery mildew-specific resistance gene diversity are indicated in *bold type*; *E* and *I* stand for Extensive and Intensive farming method, respectively. *DGER* Direction Générale de l'Enseignement et de la Recherche, *INRA* Institut National de la Recherche Agronomique, *INA-PG* Institut National Agronomique Paris-Grignon, *BRG* Bureau des Ressources Génétiques

from the four- to five-leaf growth stage (Lebsock and Briggle 1974), and its identification depended mostly on intermediate resistance reactions.

The isolates were maintained on detached primary leaf segments of specific varieties in order to avoid accidental contamination. They were regularly tested on the differential host set composed of 15 differential varieties corresponding to ten resistance genes alone or in combination (McIntosh *et al*. 1998) (Table 1) in order to detect any contamination or possible evolution. Before inoculation, they were multiplied on detached primary leaf segments of cv. Barbee, which does not carry any identified resistance gene.

For each tested line the first primary leaf of 10-day-old seedlings was cut into 2-cm-long segments (Le Boulc'h *et al.* 1995). The segments were placed on 5 g l^{-1} water agar supplemented with 30 mg l^{-1} benzimidazole (Sigma B-9131) in compartimentalized clear polystyrol plates (Limpert *et al.* 1988). Each compartment received a set of 11 leaf segments that were inoculated using a tower adapted for the plates. For each inoculation, two compartments containing the leaf segments of 20 different lines plus 1 susceptible and 1 resistant control (depending upon the isolate tested) were inoculated with one isolate. The experiment was replicated four times for each line with each isolate. After 10 days of incubation (18°C, continuous light, 7 µE m^{-2} s^{-1}), disease intensity was scored on each leaf segment using a 0–9 scale based on the number of colonies and the intensity of sporulation (0 = no visible symptoms; 9 = maximal sporulation). The scoring classes were then clustered as R (Resistant: 0–2), I (Intermediate: 3–5) and S (Susceptible: 6–9).

Statistical analysis

Test of sampling effect

To establish whether the samples of short lines could be considered as representative of the populations or not, we compared our study to a previous one by Le Boulc'h *et al.* (1994) on earlier generations of three extensive populations (generations 6 and 8 for TOE and VVE, 4 and 6 for LRE). For each gene that was common between the two studies, the homogeneity of the samples was tested by chi-square (SAS 1988, CATMOD procedure). The model consisted of the effect of the population origin (LRE, TOE and VVE) and of the year within origin (years 6, 8, 10 for TOE and VVE, 4, 6 and 8 for LRE). As the *Pmx* resistance factor had been confused with *Pm6* in the previous study, the sum of the frequencies of *Pmx* and *Pm6* obtained in our study was considered for the

Table 1 Reaction of 13 wheat differential varieties/lines carrying known powdery mildew resistance genes and of the parent C6.2.4 carrying the *Pmx* resistance factor after inoculation with the nine selected isolates of *Blumeria graminis* f.sp. *tritici*

Differential hosts	Resistance genes	Powdery mildew isolate no.								
		O	BZ	CP	96.8	96.20	DB	93.27	93.6	95.42
Ulka	*Pm2*	S[b]	S	S	S	R	S	R	R	R
Chull	*Pm3b*	R	R	R	R	R	S	R	R	R
Khapli	*Pm4a*	S	R	S	R	R	R	S	S	S
VPM	*Pmb*[a]	S	R	S	R	R	R	S	S	R
Aquila	*Pm5*[a]	S	R	S	S	R	S	S	S	S
Tp114 × st2	*Pm6*	I	S	I	R	S	S	S	S	S
Clement	*Pm8*	S	R	R	R	S	R	S	R	R
Aristide	*Mlar*[a]	S	S	I	S	S	S	R	S	S
Talent	*Tal*	S	S	S	S	S	I	S	I	S
Slejpner	*Pm2* + *Pm8*	S	R	R	R	R	R	R	R	R
Brock	*Pm2* + *Tal*	S	S	S	S	R	I	R	R	R
Mission	*Pm4b*[a] + *Pm5*[a]	S	R	S	R	R	R	S	S	R
C6.2.4	*Pm5b*[a] + *Pmx*[a]	R	R	S	S	R	I	S	S	S
Rendez-vous	*Pm2* + *Pm4b*[a] + *Pm6*	I	R	I	R	R	R	R	R	R

[a] Resistance genes postulated in the parents of the PA pool
[b] R, Resistant; I, intermediate; S, susceptible

comparison (noted *Pmx*/6). *Mlar* was not taken into account because this gene was not clearly identified in the previous study. The frequency of susceptible genotypes (with no postulated resistance genes) was also considered for the comparison. We used a factorial correspondence analysis (FCA, Benzecri 1973) to synthesize the results for all of the genes considered.

Evolution of specific resistance gene frequencies

For each gene and combination of genes (0, 1, 2, 3 or more genes), analysis of variance with site, farming condition and interaction effects were performed using the CATMOD procedure. The population of Châlons-sur-Marne (CHA) was excluded from this analysis.

The gene and genotype frequencies of the populations were compared by Fisher exact tests. When the tests were significant at the 5% level, pairs of populations were compared, and the level of significance for each comparison was set to a value of 0.05 / [n(n-1)/2], with n the number of populations, which is a very conservative test. The same test was used to compare the evolution of gene and genotype frequencies between PA0 and the seven populations pooled together. FCA was performed on all genes. Linkage disequilibria were tested at the whole pool level and within each population.

Genetic differentiation

Multigenic estimations of the Wright parameter *Fst* were calculated on the basis of two alleles, resistance and susceptibility, for each gene. Temporal *Fst* describes the comparison between the initial population and the final ones, and spatial *Fst* the comparison between two different final populations. In a previous study based on neutral RFLP markers, Enjalbert *et al.* (1999a) computed *Fst* values for PA0, two populations common to our study (LRE, TOE), and one other population (Le Moulon extensive, LME). As they used larger samples (80 lines per population) than we did, any direct comparison between the two studies was open to question. Hence, we used a bootstrap procedure (Efron and Tibshirani 1982) to obtain a distribution of *Fst* based on the RFLP data but computed with a sample size equivalent to the one we used for resistance gene estimation.
Estimation of *Fst* and linkage disequilibria were computed with GENEPOP software (Raymond and Rousset 1995). All other statistical analyses were performed using SAS software (1988).

Results

Test of sampling effect

The analysis of chi-square variation carried out on each resistance gene and the "susceptible" genotype (lines with no postulated resistance gene) revealed a highly significant effect of sample origin (LRE, TOE, VVE) ($P<0.0001$ for *Pm4b*, *Pmx*/6 and *Pm2*; $P = 0.02$ for the "susceptible" genotype; but not for *Pm8*; data not shown). The year-within origin chi-square was never found to be significant at the 5% level, which indicated that the populations have not evolved much during the last four generations and that the samples of short lines were homogenous with the samples of plants taken at random from previous generations. Consequently, the correspondence analysis carried out on the nine samples and PA0 (Fig. 2) showed a good grouping of the samples by their population origin. The sample of short plants was thus representative of the variability for specific resistance towards powdery mildew in LRE, TOE and VVE. The other populations could not be similarly tested, but we assumed that the short plants were representative there also.

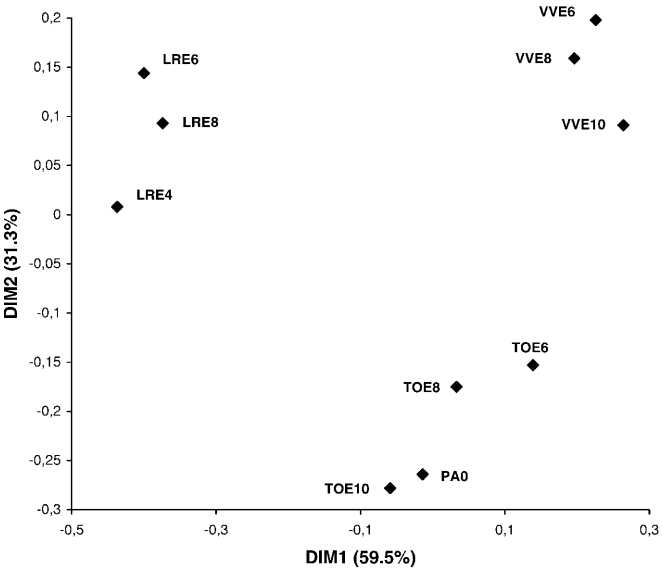

Fig. 2 Factorial correspondence analysis carried out on powdery mildew resistance gene frequencies over three generations for three wheat populations subjected to dynamic management and the initial PA0 population. The *number* after the population name indicates the generation

Evolution of specific resistance gene frequencies

In contrast to expectations after a pyramidal cross, the initial population PA0 differed quantitatively from the parents for specific resistance gene and genotype frequencies (Table 2). The frequency of susceptible lines in PA0 was less than half that in the parents, which was mainly due to an increase in *Pm4b* frequency.
Genotype frequencies in PA0 and in the seven populations pooled together (Table 2) were not significantly different. With respect to the resistance genes, the only significant change was observed for *Pm2*, which was absent in the initial population and present on average in 12% of the lines after 10 years of cultivation. *Pm6*, which was absent in PA0, could also be detected in the final pooled populations, but at a lower frequency than *Pm2*. The presence of *Pm2* and *Pm6* certainly resulted from migration processes because those two genes were and are currently very frequent in French cultivars (Zeller *et al.* 1993; Doussinault 1994). None of the parents harboured more than one specific resistance gene. In PA0, 13% of the lines had two genes (Table 2). Even if not significant, the frequency of lines with more than one gene increased in the seven pooled populations, and new genotypes, accumulating three genes, were detected after 10 years of cultivation even though they were absent in PA0.

Considering each resistance gene separately, we did not detect any major change in frequencies at the global level of the DM populations, but the situation differed at the level of each population (Table 3). Except for TOE, all the populations had diverged from PA0 (Fig. 3). For all genes except the rare ones (*Pm6* and *Pm8*), at least one population significantly differed from PA0, and at least two final populations significantly differed from each other, indicating differentiation between the DM populations (Table 3). The two populations from Toulouse, particularly TOE, were the closest to PA0, with no *Pm2*, *Pm6* and *Pm8* genes detected and a *Pm4b* frequency not different from the one observed in PA0. In contrast, the two Vervins populations (VVE and VVI) strongly diverged from PA0 and from each other, particularly for *Pm4b* frequency. The other pairs of populations cultivated at a similar site (TOE and TOI, LRE and LRI) were less divergent. These observations were confirmed at the multigenic level by *Fst* values estimated on powdery mildew resistance gene data for each pair of final populations (Table 4).

Considering multilocus genotypes, the DM populations did not diverge for the frequency of lines with only one resistance gene, and no population had significantly differentiated from PA0 for this trait. TOE was the only population for which only single specific resistance genes were detected. For the other populations, the frequency of lines with more than one resistance gene was

Table 2 Resistance gene and genotype frequencies in the parents, the initial pool (PA0) and the seven pooled dynamic management populations (DM) after 10 years of cultivation

Gene or genotype	Parents	PA0	DM	Fisher's exact test probability (PA0-DM)
Pm4b	12.5 (2[c])	38.6	31.2	0.343
Mlar	18.75 (3)	10.0	16.4	0.283
Pmx	6.25 (1)	14.3	15.6	1
Pm2	0.00	0.00	12.2	0.001
Pm6	0.00	0.00	4.8	0.088
Pm8	0.00	1.4	0.9	1
Susceptible	62.5 (10)	20.3	21.3	1
0–1 gene[a]	0.0	26.1	17.9	Not tested
1 gene	37.5 (6)	40.6	39.2	0.879
2 genes	0.0	13.0	17.6	0.537
3 genes	0.0	0.0	4.0	0.161
>1 gene[b]	0.0	13.0	21.6	0.177

[a] Lines for which the presence of a gene was hypothetical
[b] >1 gene corresponds to the sum of frequencies of genotypes with 2 and 3 postulated genes
[c] Number of parents with a given gene or genotype is indicated in brackets

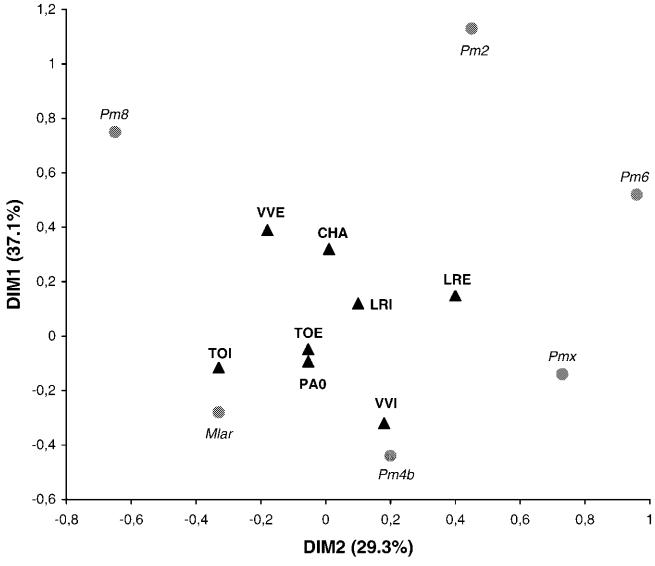

Fig. 3 Correspondence analysis carried out on a contingency table regrouping the results for the six specific powdery mildew resistance genes in the seven wheat populations after ten generations of multiplication and the initial PA0 population. *Black triangles* wheat populations, *grey circles* powdery mildew resistance genes

Table 3 Powdery mildew resistance gene and genotype frequencies (%) in the initial pool PA0) and the seven wheat populations after ten generations of multiplication with no human selection

Gene or genotpye	PA0	CHA	LRE	LRI	TOE	TOI	VVE	VVI
Pm4b	38.6 B[b]	20 A,B	29.4 B	33.3 B	35 B	26.7 B	0 A	76.2 C
Mlar	10 A,B	20 A,B,C	5.9 A,B	0 A	0 A	46.7 C	12.5 A,B	28.6 B
Pmx	14.3 A,B	13.3 A,B,C	47.1 C	11.1 A,B	10 A,B,C	0 A,B	0 A	28.6 B,C
Pm2	0 A	33.3 C	17.7 C	11.1 B,C	0 A,B	0 A,B	18.8 C	4.8A,B,C
Pm6	0 A	0 A,B	11.8 B	11.1 B	0 A,B	0 A,B	6.3 A,B	4.8 A,B
Pm8	1.4 A	0 A	0 A	0 A	0 A	0 A	6.3 A	0 A
Susceptible	20.3 A,B	20 A,B	17.7 A,B	22.2 A,B	20 A,B	13.3 A,B	50 B	4.8 A
0–1 gene[a]	26.1	20.0	11.8	27.8	35.0	20.0	12.5	4.8
1 gene	40.6 A	33.3 A	29.4 A	38.9 A	45 A	53.3 A	31.3 A	38.1 A
2 genes	13 A	26.7 A,B	23.5 A,B	5.6 A	0 A	13.3 A,B	6.3 A	47.6 B
3 genes	0 A	0 A,B	17.7 B	5.6 A,B	0 A,B	0 A,B	0 A,B	4.8 A,B
>1 gene	13 A,B	26.7 A,B,C	41.2 B,C	11.1 A,B,C	0 A	13.3 A,B,C	6.3 A,B,C	52.4 C

[a] Lines for which the presence of a gene was hypothetical. Differences between frequencies were not tested
[b] Populations with different letters were found to be significantly different for the frequency of a given gene or genotype ($P<0.0017 = 5/28$)

Table 4 Multigenic differentiation parameters (*Fst*) estimated on powdery mildew resistance gene data between all pairs of final populations (after ten generations of cultivation), and between the initial population (PA0) and each final population

[a] Values for temporal *Fst* are reported in italics

Wheat populations	Wheat populations						
	PA0	CHA	LRE	LRI	TOE	TOI	VVE
CHA	*0.084*[a]						
LRE	*0.157*	0.049					
LRI	*0.002*	0.024	0.075				
TOE	*0.000*	0.080	0.119	0.000			
TOI	*0.098*	0.083	0.228	0.141	0.171		
VVE	*0.165*	0.035	0.220	0.096	0.186	0.156	
VVI	*0.156*	0.212	0.167	0.188	0.193	0.209	0.408

Table 5 Paritioning of variance realized on the powdery mildew resistance frequencies in the six wheat populations from Le Rheu, Toulouse and Vervins after ten generations of multiplication

Effect		Gene or genotype										
		Pm4b	*Mlar*	*Pmx*	*Pm2*	*Pm6*	*Pm8*	Susceptible	1 gene	2 genes	3 genes	> 1 gene
Site	Chi2	2.46	***10.04***	***11.25***	***8.65***	***6.59***	0	0.15	2.86	***7.1***	5.74	***11.13***
	P value	0.2923	0.0066	0.0036	0.0132	0.0371	1	0.9292	0.2389	0.0288	0.0565	0.0038
Farming method	Chi2	***37.79***[a]	0.91	0.3	0	0	0	***4.22***	0.49	3.67	0.39	2.01
	P	0	0.339	0.5816	0.9905	0.9995	0.993	0.0401	0.4848	0.0554	0.53	0.1562
Interaction	Chi2	***29.75***	***14.83***	***16.73***	1.98	0.04	1.07	***7.69***	0.07	***12.22***	2.32	***16.46***
	P	0	0.0006	0.0002	0.3717	0.9794	0.5867	0.0214	0.9659	0.0022	0.3127	0.0003

[a] Bold italics indicate significant chi-square (Chi2)

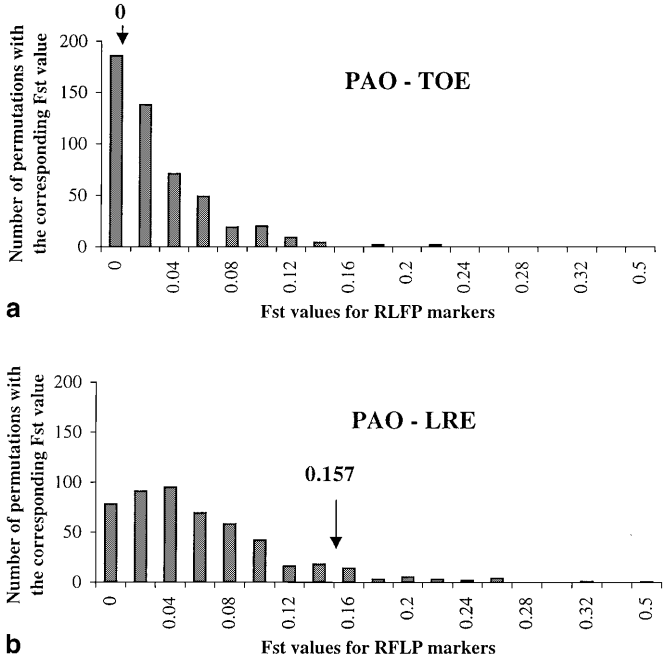

Fig. 4a, b Distribution of temporal *Fst* values estimated on RFLP data by the bootstrap procedure on 6 random RFLP loci. *Arrows* indicate the position in the distribution of temporal *Fst* calculated for the corresponding population pair on specific powdery mildew resistance gene data. Population comparison: **a** *PA0-TOE Fst* value calculated on specific powdery mildew resistance gene data was not within the upper 5% of the bootstrap values for RFLP data. **b** *PA0-LRE Fst* value calculated on specific powdery mildew resistance gene data lay within the upper 5% of bootstrap values for RFLP data

maintained or increased, as for LRE ($P<0.1$) and VVI ($P<0.01$) (Table 3). For LRE, the most frequent combination was observed between *Pm2* and *Pmx*, whereas in LRI it was between *Pm2* and *Pm6*. In those two populations, positive linkage disequilibria were significant between *Pm2* and *Pmx* ($P = 0.02$) or *Pm2* and *Pm6* ($P = 0.04$). No other significant linkage disequilibrium was found in any population including PA0.

The partitioning of variance for each gene and genotype revealed that significant site or farming-method effects were always associated with a (site × farming-method) interaction, except for *Pm2* and *Pm6* for which only the site effect was significant (Table 5). Site effect was found significant in more cases than farming condition effect.

The distribution of temporal *Fst* values estimated by the bootstrap procedure on RFLP data for TOE and LRE, the two populations from the present study also investigated for RFLP markers, are presented in Fig. 4. In the case of TOE, the *Fst* value calculated on powdery mildew resistance was not within the upper 5% of the bootstrap values for RFLP data, whereas it was in the case of LRE.

Discussion

Our results show that for seven populations of the wheat DM network the diversity of powdery mildew-specific resistance genes was maintained: all of the genes detected in the initial population were present in the global final

population, and no significant change had occurred in their frequencies. In contrast, diversity strongly decreased in each population, so that some genes could no longer be detected in some of the populations (*Mlar* in LRI and TOE, *Pm4b* in VVE, *Pmx* in TOI and VVE). Thus, maintenance of diversity occurred through a strong differentiation of the populations. Each population had diverged more (VVI, VVE) or less (TOE) from the initial population, and most of the final populations were also different from each other. Farming method seemed to have no global effect on the structure; this effect was only detected for *Pm4b* and was due to the strong differentiation observed between the VVE and VVI populations.

Previous studies carried out on different traits have shown similar results. After a few generations of cultivation, differentiation between the populations of the network was observed for morphological and agronomical traits (David *et al.* 1992), endosperm storage proteins (Pontis 1992) and protein profiles of leaf extracts (revealed by two-dimension electrophoresis, David *et al.* 1997). The between-population differentiation compensated for the loss of variability observed within some of the populations for most of the traits except those involved in competition between plants (e.g. an undesirable general increase in adult-plant height was observed in all the populations derived from the selfing pools; Le Boulc'h *et al.* 1994). Recent studies on molecular markers (RFLP) revealed that after 10 years of cultivation neutral genetic diversity was maintained: even though allelic frequencies were strongly modified in the different populations, at the scale of the network the allelic richness was maintained (Enjalbert *et al.* 1999a).

The expected temporal *Fst* value due to random drift after ten generations for an idealized population (Falconer 1989) of approximately 5000 individuals (corresponding to the demographic size of DM populations) is 0.002 (Enjalbert *et al.* 1999a). For all the populations studied by Enjalbert *et al.* (1999a), the actual values were found to be higher than 0.002, which indicates that the populations did not behave as idealized populations. The temporal *Fst* value obtained on RFLP data showed that TOE was the population that had evolved the least compared to PA0 (*Fst* = 0.015, Enjalbert *et al.* 1999a), which was also true for specific resistance gene data (*Fst* = 0.000; Table 4, Fig. 4). In this population powdery mildew-specific resistance genes did not evolve differently than neutral markers. The LRE population was very different. Indeed, LRE proved to have strongly diverged from PA0 (*Fst* = 0.036, Enjalbert *et al.* 1999a). As the *Fst* value calculated on specific resistance genes' data (*Fst* = 0.157, Table 4, Fig. 4) was superior to the 5% threshold (*Fst* = 0.150) of the bootstrap distribution, we can suppose that in LRE, selection played a role in the evolution of powdery mildew-specific resistance gene frequencies.

Among the three populations studied with RFLP markers, LRE was found to have diverged the most from PA0 (Enjalbert *et al.* 1999a). The high *Fst* value obtained on RFLP data (*Fst* = 0.036) and some information on the population management in Rennes (Trottet, personal communication) led us to conclude that the Rennes population may have experienced a bottleneck. If we consider the *Fst* distribution in LRE as a reference for variation in allelic frequencies of neutral loci in a population having strongly diverged, the temporal *Fst* values obtained for VVE (*Fst* = 0.165) and VVI (*Fst* = 0.156) could be explained either by selection for powdery mildew-specific resistance in those populations or by the existence of a bottleneck in the history of the populations (the two phenomena are not mutually exclusive), while the low *Fst* value obtained for LRI suggests that the evolution of this trait was mainly due to genetic drift in this population. In the case of CHA and TOI, the intermediate temporal *Fst* values obtained with specific resistance genes data (*Fst* = 0.084 and 0.098, respectively) did not allow us to infer whether selection played a role or not in the evolution of powdery mildew-specific resistance genes frequencies.

Toulouse is a site where powdery mildew pressure was low, whereas it was rather strong in Rennes. It is then very interesting to find that specific resistance genes were not subjected to selection in TOE, whereas they seemed to be in LRE. Discussion on the other populations is a little more critical. Indeed, as we have no idea of the evolution of neutral diversity in these populations, even though high *Fst* (>0.15) values could be interpreted as a result of selection in most of the cases, the hypothesis of a strong bottleneck can not be ruled out. The case of the Vervins populations is a good illustration of the problem. The two populations (VVE and VVI) showed strong and similar temporal *Fst* values (0.165 and 0.156, respectively). Because in VVI the frequencies of all the observed resistance genes had increased, we thought that a positive selection for resistance could be the main factor involved in the evolution. This result was not really unexpected since Vervins had been chosen as representing the site of the network with the strongest powdery mildew pressure. For VVE, the stochastic and drastic changes in frequency observed for the different resistance genes (38–0% for Pm4b, 14–0% for *Pmx*) and the increase in susceptible genotype frequencies (20–50%) led us to the conclusion that VVE had certainly experienced an important bottleneck. This event took place before the sixth generation, as the frequencies observed by Le Boulc'h (1994) were similar to the ones found in the present study. The relative history of VVI and VVE would require further investigations based on neutral markers. A lesson drawn from the present study is that any change in the frequency of neutral markers should be investigated for any specific population as a tool to help in interpretating the data of any other trait in the population considered. Uncontrolled migration appears to be a factor to be considered in the evolution of DM populations, as resistance genes that were absent in the initial pool reached non-negligible proportions in some of the final populations. Caution should be taken to reduce those phenomena (even if it seems illusory to prevent them completely), as controlled gene flow between populations is essential for a long-term conservation of genetic variability (Olivieri e*t al.* 1990).

In the case of powdery mildew, resistance gene diversity was maintained through different mechanisms, depending on the population considered. In some populations, resistance genes seemed to evolve the same way as neutral markers, and in others, they seemed to be selected, even though it was not possible to evaluate the relative importance of drift and selection involved in the evolution of the frequency of each gene in each population. Managing a sufficient number of populations, located in sites with varying degrees of selection pressures (from no selection to strong but different selection pressures), seems to be a good solution, at least on the short term (10 years is a short period for a genetic resources conservation programme) to allow for the maintainance of such a variability, even if some populations can experience strong bottlenecks.

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