Theor Appl Genet (2007) 114:787–802
DOI 10.1007/s00122-006-0477-x

ORIGINAL PAPER

Strong linkage disequilibrium near the selected *Yr17* resistance gene in a wheat experimental population

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Received: 20 June 2006 / Accepted: 24 November 2006 / Published online: 20 December 2006

Abstract Dynamic management (DM) is a method of genetic resources conservation that aims at maintaining evolutionary process in subdivided populations cultivated in contrasted environments. Such populations are often submitted to strong natural selection as it was the case for experimental wheat populations maintained under DM. Understanding impacts of selection on genetic diversity around selected genes is necessary for the middle-term maintenance of genetic variability in DM populations. Evolution of diversity at six neutral markers located near the yellow rust resistance gene *Yr17* has been studied for the parental lines and for generations 1, 5, 10 and 17 in one of the DM populations. *Yr17* provided complete resistance to yellow rust in France until 1997 and thus was suspected to be under strong selection. The gene is located on a fragment introgressed in winter wheat from a wild species. The presence of the gene was estimated using a marker closely related to the gene. We showed that the *Yr17* gene has been selected between generations 5 and 10. Generally, selection tends to reduce diversity around selected genes, generating linkage disequilibrium (LD) between a gene and adjacent markers. Here, the major effect of the *Yr17* gene selection was a reduction of multilocus diversity and the maintenance of strong pre-existing LD in the zone surrounding the gene for a distance of 20 cM. As expected, the presence of the exogenous introgression was responsible for restrictions to recombination which contributed to the maintenance of strong correlations between loci. However, we found a noticeable number of recombinations around the gene indicating a progressive incorporation of the fragment into the wheat genome.

Communicated by M. Sillanpää.

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Introduction

Dynamic management (DM) is a method for in situ conservation of genetic resources that aims to maintain adaptive potential in genetically heterogeneous populations. These populations are grown for many generations in a multilocation network and are subjected to evolutionary pressures (natural selection, genetic drift, mutation, migration and recombination) present in their usual agro-ecosystem (Henry et al. 1991). Genetic differentiation for adaptive traits in populations is a major mechanism of DM programs and can be due to natural selection and/or genetic drift. The major role of natural selection in shaping genetic diversity of natural populations has been reviewed by Merilä and Crnokrak (2001). To date, few experimental data are available. Experimental populations, where the initial stage is fully known and several generations available, are useful to study the effect of natural selection and the link between diversifying selection and genetic differentiation for quantitative traits (Porcher et al. 2004).

Rapid differentiation of populations under natural selection has been observed in two long-term programs of experimental crop populations. The first program has been conducted since 1928 on barley populations (*Hordeum vulgare* L.) in California (Allard 1988). The goal of this experiment was to generate improved genotypes by cultivating, over generations, a composite population subjected to natural selection without intentional human selection. The experiment showed a rapid evolution of the population. In particular, competition between individuals led to an improvement of some quantitative traits such as population mean grain yield, spike weight, number of seeds per spike, earliness and resistance to powdery mildew disease (Allard 1988; Ibrahim and Barrett 1991). Secondly, a DM program has been conducted in France since 1984, which is based on winter wheat (*Triticum aestivum* L.) composite populations distributed in a multisite network (Henry et al. 1991). Since the beginning of the experiment, each wheat sub-population has been cultivated in isolated conditions and without deliberate migration. Previous studies on the DM populations reported rapid spatial and/or temporal differentiations of the sub-populations, interpreted as a response to natural selection pressures (David et al. 1992; Le Boulc'h et al. 1994; Paillard et al. 2000a, b; Goldringer et al. 2001).

In the context of genetic resources conservation, it is important to understand the impact of natural selection on genetic diversity within the different populations. Population genetics theory predicts that selection on one gene can (1) reduce genetic variability at linked neutral loci, and (2) generate linkage disequilibrium (LD) between the locus subjected to selection and linked loci, i.e. the so-called hitch-hiking effect (Maynard Smith and Haigh 1974; Ohta and Kimura 1975; Kaplan et al. 1989). Linkage disequilibrium is reduced by recombination and mutation but in predominantly selfing species such as wheat, recombination between heterozygous fragments is rare and may have little effect in breaking associations generated by selection. Thus more extensive LD is expected than in outcrossing species (Nordborg 2000; Flint-Garcia et al. 2003).

In this article, we report the impact of selection on diversity near a cluster of three rust resistance genes in a DM population cultivated at Le Moulon. The linked resistance genes are *Yr17* (for resistance to yellow rust caused by *Puccinia striiformis* West. F. sp. *tritici*), *Lr37* (for resistance to leaf rust caused by *Puccinia triticina* Eriks) and *Sr38* (for resistance stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.). In a previous work, Paillard (1999) showed that the *Yr17* gene was present in four among the 16 parental lines of the DM population, and that the frequency of the gene increased between generation 0 and 10 in several populations of the wheat DM network. This is why we suspected that *Yr17* was under strong selection in most of the DM populations. Meanwhile, yellow rust was present in most areas in the North of France and the gene provided a significant increase in adult phenotypic resistance assessed in three consecutive years in three different sites (Paillard 1999). The three resistance genes (*Yr17*, *Lr37* and *Sr38*) were initially introgressed in the winter wheat variety 'VPM1' from *Aegilops ventricosa* Ces. (Maia 1967) and are located on a 2NS/2AS translocation (Bariana and McIntosh 1993, 1994). Helguera et al. (2003) suspected a strong restriction to recombination between 2AS and 2NS of the *Yr17-Lr37-Sr38* introgression. Introgression of genomic segments from alien species severely restricts recombination. Minimal recombination has been found for disease resistance loci introgressed from wild relatives to hexaploid wheat (Naik et al. 1998; Seyfarth et al. 1999; Neu et al. 2002; Helguera et al. 2003). Indeed, related wild species are widely used as sources of new resistances in wheat to counter the continuous evolution of pathogen populations (for a review, see Friebe et al. 1996; Bommineni and Jauhar 1997; Fedak 1999). In this context, selection for a resistance gene might have a strong impact on genetic diversity near the gene and pattern of linkage disequilibria would locally be maintained a long time after selection.

The objectives of this study were: (1) to verify if the *Yr17* resistance gene has been selected in the studied population and to characterize the conditions of this selection (intensity, duration); (2) to determine the impact of such a selection on neutral diversity near a selected gene located in an introgressed fragment; to assess the potential of DM for incorporating within the wheat genome introgressed alien fragments.

Materials and methods

Plant material

In 1984, a winter wheat DM network was created by the Institut National de la Recherche Agronomique (INRA) to study the mechanisms of adaptation and evolution of experimental populations in contrasted environments. The population studied called PA was created by four generations of pyramidal crosses (Fig. 1) among 16 French wheat varieties chosen for their agronomical value and the complementarities of their agronomical traits (resistance, plant height, etc.;

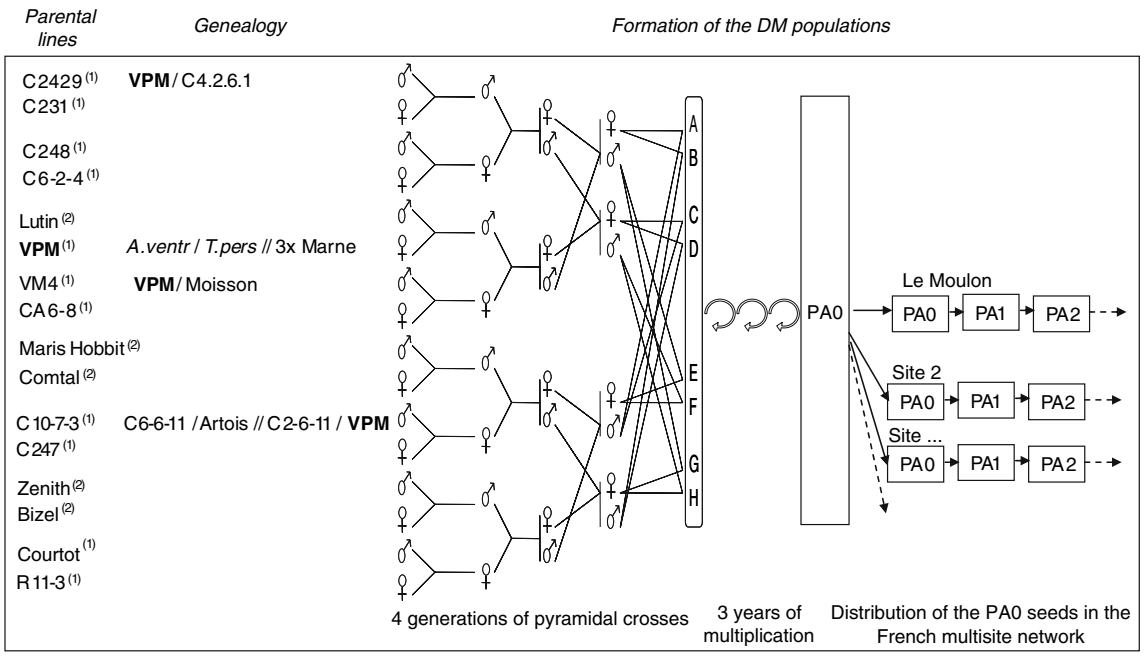

Fig. 1 Presentation of the parental lines and formation of the DM populations. Genealogy is given for parental lines carrying the *Yr17-Lr37-Sr38* fragment only. (*A. ventr.*:*Aegilops ventricosa*; *T. pers.*:*Triticum persicum*). Origin of the parental lines: (1) INRA, (2) commercial varieties from the French catalog

Thomas et al. 1991). In 1984, after 3 years of multiplication to increase seed set, this composite population, called PA0, was sown in a French multisite network. In this study we focused on the population cultivated under DM at Le Moulon (near Paris) under low-input farming condition (low nitrogen and no fungicide). The population was cultivated in a 100 m^2 plot isolated from other wheat cultures. Each year, the whole plot was harvested in bulk, a sample of 10,000 seeds was sown to constitute the next generation and 500–1,000 grams of seeds were stored in a cold room (Henry et al. 1991). We studied genetic diversity among the 16 parental lines and 159 individuals from generation 1 (PA1) separated from parental lines by eight generations of intercross and multiplication (Fig. 1), 130 individuals from generation 5 (PA5), 125 individuals from generation 10 (PA10) and 170 individuals from generation 17 (PA17).

Molecular diversity

Molecular diversity around the Yr17 resistance gene

DNA of the 16 parents, and all individuals sampled in PA1, PA5, PA10 and PA17 was extracted from young leaves following a protocol adapted from Dellaporta et al. (1983). Presence of the *Yr17* resistance gene was assessed through a dominant *SCAR* marker (*SC-Y15*) 0.8 cM from this resistance gene (Robert et al. 2000a). The *SC-Y15* fragment was amplified following a protocol adapted from Robert et al. (2000a). The amplification fragments were separated on 0.8% agarose gels containing ethidium bromide and were visualized with UV light.

Five microsatellite markers (*Xgwm* markers) developed by Röder et al. (1998) and one marker (*Xwmc177*) developed by Gupta et al. (2002) on the short arm of the chromosome 2A were chosen to cover a 60 cM segment around the *Yr17* gene (Fig. 2). Microsatellite markers were located from public mapping data available on ITMI population (provided by P. Leroy, INRA) using Mapmaker software, and *SC-Y15* was located approximately close to *Xgwm636* and *Xgwm512* according to data provided by F. Dedryver (personal communication). Previous studies (Bariana and McIntosh, 1993; Helguerra et al. 2003) located the introgression in the distal part of the short arm of the chromosome 2A, but its delimitation is still inaccurate. We located the lower limit of the introgression approximately between *Xgwm512* and *Xgwm359* according to data provided by F. Dedryver. Except *Xgwm512* that was dominant, all the markers showed size polymorphism among the 16 parents. For these markers, forward primers were modified with a M13

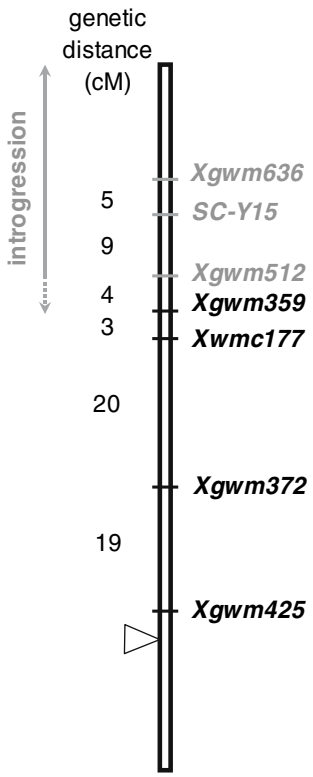

Fig. 2 Map position of the microsatellite markers and the introgression on the chromosome 2A. Microsatellite markers were located from public mapping data on ITMI population. *SC-Y15* was positioned approximately close to *Xgwm636* and *Xgwm512*. The markers belonging to the introgression are indicated in *gray letters*. (Personal communication: F. Dedryver)

extension according to Boutin-Ganache et al. (2001). PCR amplifications were performed in the presence of fluorescent-labelled M13 tailed. Amplification products were obtained with minor modifications to the protocol of Röder et al. (1998). Amplification products, loaded on 6.5% denaturing polyacrylamide gels, were separated on a LiCor automated DNA sequencer (Licor Biosciences), and analyzed with the OneDscan software (version 2.03, Scanalytics). The dominant marker *Xgwm512* was amplified from 200 ng genomic DNA in 20 µl volumes using 1.5 mM MgCl2, 200 µM dNTPs, 0.5 µM of each primer, and 1 U Taq polymerase. Cycling conditions were 3 min at 94°C followed by 35 cycles (1 min at 94°C, 1 min at 60°C, 2 min at 72°C), and 10 min at 72°C. The amplification fragments were separated on 0.8% agarose gels containing ethidium bromide and were visualized with UV light.

Molecular diversity of the whole genome for the parental lines and PA17

To assess genetic diversity of the whole wheat genome, nineteen microsatellite markers (*Xgwm*) developed by Röder et al. (1998) and one marker (*Cfd71*) developed by Guyomarc'h et al. (2002) which amplified two loci, were chosen to cover the 21 wheat chromosomes. All microsatellite markers were chosen polymorphic within the parents. Genetic diversity at these markers was analyzed among the 16 parents and 332 individuals of PA17, and constituted a reference for the evolution of diversity at the whole genome level. PCR was performed in a volume of 10 µl. The reaction mixture contained 1.5 mM $MgCl_2$, 500 nM of each primer, 200 µM of each deoxynucleotide, 40 ng of template DNA, and 0.2 unit Taq polymerase. Cycling conditions were 3 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 50–60°C (depending on the primer, and according to Röder et al. (1998) and to Guyomarc'h et al. (2002), 30 s at 72°C, and 10 min at 72°C. Amplification products were separated on a ABI 3100 semi-automated sequencer, and analyzed with GeneScan software (Eurofins). PCR amplification, fragment separation and amplification analyses were realised at Clermont-Ferrand INRA laboratory.

Data analyses

Temporal evolution of allele frequencies

For each of the seven markers located around the *Yr17* gene, we estimated the allelic frequencies in the samples of the different generations studied. We considered temporal variations of allelic frequencies between generations as follows: parents versus PA1, PA1 versus PA5, PA5 versus PA10, PA10 versus PA17, Parents versus PA17. Temporal variance of allelic frequencies was estimated by the standardized variance (F_c) at each locus l with K alleles (Nei and Tajima 1981):

$$F_{c,l} = \frac{1}{K}\sum_{k=1}^{K}\frac{\left(p_{ki}-p_{kf}\right)^2}{\left(\frac{(p_{ki}+p_{kf})}{2}-p_{ki}\times p_{kf}\right)}$$

where p_{ki} (p_{kf}) were the frequencies of allele k in the sample of the initial (final) population.

The multilocus F_c was calculated as the weighted mean of the single locus $F_{c,l}$ values and was used to estimate the genetic effective population size (N_{eg}) assuming that the variance of allele frequency due to drift from parents to offspring, directly depends on N_{eg} (Waples 1989):

$$N_{eg} = \frac{t}{2\left(\hat{F}_c - \frac{1}{S_0} - \frac{1}{S_t}\right)},$$

where t is the number of generations between the two populations studied, and S_0 (S_t) was the sample size at the initial (final) generation.

F_c and N_{eg} between the parents and the population PA17 were also estimated using the 21 markers on the whole genome. Confidence intervals around N_{eg} were estimated assuming that $n\hat{F}c \big/ E(\hat{F}c)$ followed an approximate chi square distribution with n degrees of freedom ($n = \sum_l (K_l - 1)$ with K_l the number of alleles at locus l; Lewontin and Krakauer 1973).

To detect selection at a particular locus, one possibility is to look for heterogeneity in individual $F_{c,l}$ values. Several factors can modify the local effective size at a given locus, among which direct selection on the locus or hitch-hiking that will drive higher than expected the temporal variance in allele frequency of markers linked to a positively selected variant (Wiehe and Stephan 1993). Here, we used the method proposed by Goldringer and Bataillon (2004), where markers exhibiting $F_{c,l}$ values significantly higher than expected under pure drift are detected. To do so, each observed $F_{c,l}$ value for each locus located in the *Yr17* region was compared to an expected distribution derived by simulations based on the estimated genome-wide population effective size, N_{eg} (here $N_{eg} = 167$). The evolution of an ideal Wright–Fisher population of size N_{eg} was simulated with multinomial sampling of alleles, at each generation, based on their frequencies at the previous generation and starting with the observed initial allele frequencies in the experimental population. As described by Goldringer and Bataillon (2004) we assigned a P value for the estimated $F_{c,l}$ at each locus of the segment around *Yr17* corresponding to the null hypothesis, temporal allelic variations are homogeneous throughout the genome.

The previous method allowed us to detect a positive selection of the allele *ventricosa* (allele 1) at *SC-Y15* linked to the presence of the *Yr17* gene. To quantify the strength of this selection we supposed a constant coefficient of selection (s) over generation and the following selective values: $(1 + 2s)$ for the genotype [allele 1/allele 1] and [allele 1/allele 2] and 1 for [allele 2/allele 2]. Knowing the partial outcrossing rate and the frequency of the different genotypes in PA5, we computed the expected genotypic frequencies of *SC-Y15* in PA10 under the assumption of an infinite population and for different s values. Partial outcrossing rate ($t = 2.8\%$) was inferred from the residual heterozygosity at the 21 loci in PA17. The best estimate of s was the value that best fit the observed genotypic frequencies of *SC-Y15* in PA10.

Diversity analyses

Genetic diversity was calculated at each generation and at each locus as follows (Nei 1973):

$$h = \left(1 - \sum_{i=1}^{n} p_i^2\right),$$

where p_i was the frequency of allele, i and n the number of alleles at the locus.

Nonrandom association between alleles at different loci (LD) was studied using the common correlation coefficient (R) between loci and the correlation coefficient between alleles at different loci (R_{ij}). They were calculated with LINKDOS software (Garnier-Gere and Dillmann 1992) based on the estimate of LD proposed by Weir (1979):

$$\Delta_{ij} = \frac{N}{N-1}\left(\frac{T_{ij}}{N} - 2p_i q_j\right)$$

where T_{ij} is the number of times that allele i at a locus appears with allele j at another locus in the same individual, p_i is the allelic frequency of allele i and q_j the allelic frequency of allele j in the sampled population of N individuals. The correlation coefficient R_{ij} calculated from the Δ_{ij} was corrected by a coefficient for departures from random mating (Black and Krafsur 1985). To reduce the effects of rare alleles, the common correlation coefficient was calculated from correlations between alleles whose frequency was higher than 5%.

Allelic associations between the loci found in the alien introgression of wild wheat (*Xgwm636*, *SC-Y15*, *Xgwm512*) were studied using trilocus genotypes. Haplotypes were reconstituted and their frequencies were estimated from multilocus genotypic data using the ARLEQUIN software (version 2.000, Schneider et al. 2000). Since they could not be detected, heterozygotes at dominant markers *Xgwm512* and *SC-Y15* were merged with homozygotes; heterozygotes at locus *Xgwm636* generated two different haplotypes with frequencies 1:1. As the studied PA population was mainly selfing, the rate of heterozygotes was low (less than 5%) and the number of haplotypes generated by this procedure was limited.

A minimum spanning network (MSN) of haplotypes was drawn for each generation using the MINSPET program provided by ARLEQUIN 2.000. The network was computed from a distance matrix containing the number of differences between each pair of haplotypes and associated haplotypes showing only one difference. We used this approach to identify recombination events between markers located in the introgression.

Results

Detection of selection

Presence of a band at the *SC-Y15* dominant marker is supposed to be tightly correlated with the presence of the *Yr17* resistance gene (Robert et al. 2000a) belonging to the *Aegilops ventricosa* introgressed fragment. The correlation between phytopathological tests and *SC-Y15* data has been verified within the 16 parents and in PA10 by Paillard (1999). Hence we called *ventricosa* the allele corresponding to the presence of a band. The *SC-Y15 ventricosa* allele was present in four of the 16 parents: VPM (parental line in which *Yr17-Lr37-Sr38* fragment was initially introgressed) and three other parental lines (VM4, C10-7-3, C2429) which had VPM in their genealogy (Fig. 1). These four parents presented the same haplotype at the three markers close to the *SC-Y15* (*Xgwm636*: allele 9; *Xgwm512*: allele 2 specific of the four parents; *Xgwm359*: allele 4). The *SC-Y15* allelic frequencies didn't evolve monotonically across generations. Rather, variations were erratic: the *ventricosa* allele frequency increased from 0.25 within the parents to 0.36 ($CI_{95\%}$ = [0.29; 0.44]) in PA1, decreased to 0.23 ($CI_{95\%}$ = [0.16; 0.31]) in PA5, then again increased significantly up to 0.46 ($CI_{95\%}$ = [0.37; 0.54]) in PA10 and finally it decreased to 0.30 ($CI_{95\%}$ = [0.23; 0.37]) in PA17. Among the closest markers to *SC-Y15*, *Xgwm636 ventricosa* allele showed a pattern of evolution very similar to the one of the *SC-Y15 ventricosa* allele, while *Xgwm512 ventricosa* allelic frequency regularly increased until generation 10 with a significant increase between generations 5 and 10.

The effective population size between the parents and PA17 (N_{eg} = 167, $CI_{95\%}$ = [119–216]) estimated from the averaged temporal variance of allele frequencies F_c over the 21 loci was similar to the effective population size calculated previously with RFLP markers (N_{eg} (PA1–PA10) = 144, N_{eg} (PA1–PA5) = 216, N_{eg} (PA5–PA10) = 157, Goldringer et al. 2001), which is quite constant across generations. N_{eg} calculated for each interval of generations from averaged F_c over the seven markers of the *Yr17* chromosomal zone ranged from 73 to 298 (Table 1). The lower value (N_{eg} = 73) was observed between PA5 and PA10. Otherwise, N_{eg} was similar to the mean value observed on the whole genome. The low N_{eg} value between generations 5 and 10 was due to very low N_{eg} value at markers *Xgwm636*, *SC-Y15*, *Xgwm512* and *Xgwm359,* all located in or close to the introgression (Table 2). These low N_{eg} values resulted from large temporal variance in allele frequencies assessed by the parameter $F_{c,l}$. To test whether these variations were due to local selection, simulations of random evolution of allelic frequencies in a population of effective size N_{eg} = 167 were run to generate the expected distribution of individual $F_{c,l}$ under the null hypothesis of homogeneous evolution of the genome. The probability to get an $F_{c,l}$ value equal or greater than the $F_{c,l}$ observed was 0.021 for *Xgwm636*, 0.007 for *SC-Y15*, 0.018 for *Xgwm512* and 0.017 for *Xgwm359*. These results led us to conclude that a significant effect of selection had locally reduced the effective size around *Yr17* gene between generations 5 and 10. Under the assumption that selection of the *Yr17* gene is the main cause of this evolution, we estimated the value of the coefficient of selection for *SC-Y15* as s = 0.13.

N_{eg} calculated from *SC-Y15* allelic frequencies data was low for each time interval due to important changes of the *SC-Y15* allelic frequency between generations (Table 2). In some cases, N_{eg} estimated for *Xgwm512*, *Xgwm372* and *Xgwm425* markers tended towards the maximum value for N_{eg} fixed at 2,625 (the demographic effective population size) because of very low variations of allelic frequencies observed between generations especially for markers distant from *SC-Y15*.

Evolution of diversity near the resistance gene compared to the rest of the genome

As expected, microsatellite markers showed a high molecular polymorphism. Among the 21 loci located

Table 1 Genetic effective size calculated, between each generation, from mean temporal variance of allelic frequencies over the seven markers of the *Yr17* chromosome zone and confidence intervals (CI)

Interval	Number of generations	S_0 Initial sample size	S_t Final sample size	N_{eg} mean	CI (95%)
Par–PA1	8	–	159	152	(75–249)
PA1–PA5	4	159	130	298	(83–8505)
PA5–PA10	5	130	125	73	(33–134)
PA10–PA17	7	125	170	199	(80–453)
Par–PA17	24	–	170	200	(104–305)

Table 2 Genetic effective size (N_{eg}) calculated for each locus between each generation

Interval	*Xgwm636*	***SC-Y15***	*Xgwm512*	*Xgwm359*	*Xwmc177*	*Xgwm372*	*Xgwm425*
Par–PA1	127	**73**	2625	54*	611	242	259
PA1–PA5	450	**26***	2625	138	232	2625	2625
PA5–PA10	62*	**12***	19*	42*	227	2625	2625
PA10–PA17	214	**41**	2625	210	167	267	2625
Par–PA17	251	**1694**	54	97	324	267	342

The upper limit for N_{eg} was fixed at 2625, the demographic effective size of the PA population

*P value < 0.05, where P value is the probability that observed F_c was smaller than simulated F_c

on the whole genome, 86 parental alleles were detected. Five of the parental alleles were not found in PA17, whereas 31 new alleles were detected. The average number of alleles per locus was 4.1 within the parents and increased to 5.3 in PA17. The same trend was observed for the five co-dominant markers near the *Yr17* gene (*Xgwm636*, *Xgwm359*, *Xwmc177*, *Xgwm372* and *Xgwm425*) presenting microsatellite polymorphism: while 31 alleles were found for the parents, 11 new alleles were detected in PA17. The average number of alleles per locus increased from 6.2 within the parents to 8.4 in PA17.

The mean diversity of marker loci in the chromosomal zone near *Yr17* estimated at the different generations ($h_{parents}$ = 0.65; h_{PA1} = 0.62; h_{PA5} = 0.65; h_{PA10} = 0.61; h_{PA17} = 0.61) was always higher than the mean diversity found for the 21 locus in PA17 (h = 0.54). Nei diversity at a given locus depends on the number of alleles at the locus and on allelic frequencies and it is maximal for a high number of alleles at equal frequencies. The two markers (*Xgwm512*, *SC-Y15*) located close to the *Yr17* gene presented the lowest values of the Nei index over generations; however these markers had only two alleles and their values were close to 0.5, the maximal expected value. No clear tendency was found for the evolution of genetic diversity at *SC-Y15*. In particular, diversity increased from 0.34 to 0.50 between generations 5 and 10, period for which we suspected a positive selection of *Yr17*. Indeed, diversity increased because the selected *ventricosa* allele was at relatively low frequency in PA5 (0.23) and moved to 0.46 in PA10. Among all markers, only *Xgwm636* and *Xgwm359* showed a decrease of gene diversity between generations 5 and 10. Diversity at the other loci evolved in an erratic way.

In order to better characterize the impact of the gene selection on diversity, we estimated gene diversity of all individuals carrying the *ventricosa* allele at *SC-Y15* which we compared to diversity of all individuals without the *ventricosa* allele at *SC-Y15* (Fig. 3). Parents with the introgression were monomorphic (h = 0) at *SC-Y15*, *Xgwm512*, *Xgwm636* and *Xgwm359* and genetic diversity was also reduced for the *Xwmc177* and *Xgwm372* markers. At the following generations, genetic diversity of individuals with the *ventricosa* allele at *SC-Y15* was similar to diversity of individuals without the *ventricosa* allele for the most distant markers *Xgwm372, Xgwm425* and *Xwmc177*. For the three proximal markers, *Xgwm636, Xgwm512* and *Xgwm359*, genetic diversity remained very low within the individuals with the *ventricosa* allele at *SC-Y15*. Whereas genetic diversity increased in PA1 compared to the parents, indicating that multiple crosses had generated new haplotypes carrying the *ventricosa* allele at *SC-Y15*, it decreased between generations 5 and 10. In contrast, diversity of individuals without the *ventricosa* allele was high and stable over generations.

Evolution of linkage disequilibrium near the resistance gene

Linkage disequilibria were estimated between *SC-Y15* and each of the six other markers of the segment at the different generations (Fig. 4). The high values of LD for the parents were probably due to the small number of parental lines that included some related lines with identical pattern of allele associations. LD calculated for markers located in the introgression (*Xgwm636* and *Xgwm512*) were particularly high, indicating a strong structuring of the chromosomal zone around *Yr17* gene. On the other hand, LD was lower outside the introgression, with a clear disruption between *Xgwm512* and *Xgwm359*. The most distant markers (*Xwmc177*, *Xgwm372* and *Xgwm425*) showed LD values for the parents similar to those found between the 21 physically independent markers of the whole genome (mean value $LD_{WGparents}$ = 0.33). In PA1, LD values decreased for all markers as a consequence of the four generations of intercrosses and the three generations of bulk multiplication. LD values slightly decreased from generation 1 to generation 5. The two most distant markers (*Xgwm372* and *Xgwm425*) showed LD values similar to those (LD = 0.08) found in a previous study between 29 RFLP markers dis-

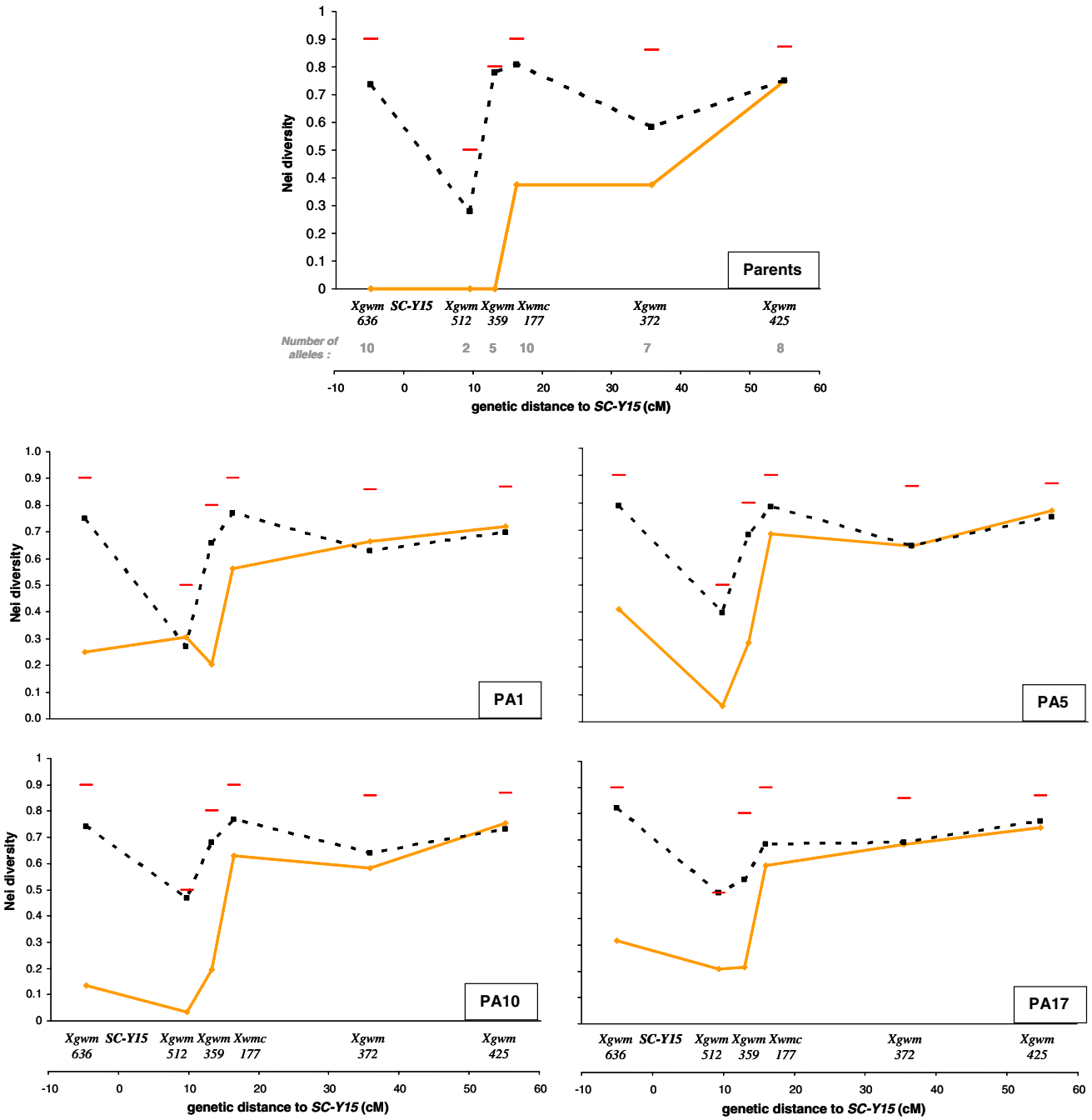

Fig. 3 Nei diversity (*h*) calculated for individuals possessing the *ventricosa* allele at *SC-Y15* (*gray solid line*) and for individuals without the *ventricosa* allele at *SC-Y15* (*dashed line*) along the chromosome 2A at each generation. *Short lines* indicate the maximal possible values at each locus depending on the number of alleles as a reference. In *gray* is indicated the number of allele at each locus

tributed over the whole genome in PA1, PA5 and PA10 (data not published) and to those found between the 21 markers of the whole genome for PA17 (mean value of $LD_{WGpa17} = 0.09$). Correlations found between *SC-Y15* and the distant markers *Xgwm372* and *Xgwm425* were not significant in PA1, PA5, PA10 and PA17. Between generations 5 and 10, correlations between *SC-Y15* and the four closest markers (*Xgwm636*, *Xgwm512*, *Xgwm359* and *Xwmc177*) increased and then, between generations 10 and 17, it tended to decrease especially between *SC-Y15* and *Xgwm636* and *Xgwm512*.

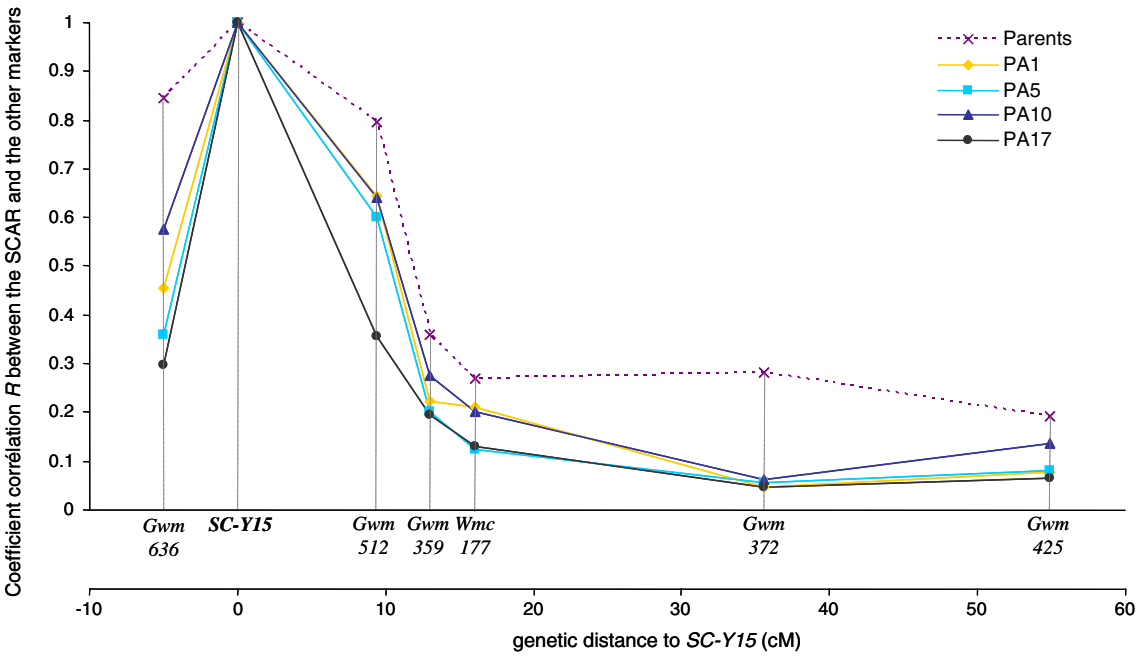

Fig. 4 Linkage disequilibrium (expressed as the common correlation coefficient *R*) between *SC-Y15* and the markers around *Yr17* gene as a function of the distance to *SC-Y15* at each generation

Table 3 Detail correlation coefficients (R_{ij}) calculated between *ventricosa* alleles of the introgression loci

Generation	Loci *Xgwm636*/*SC-Y15* *R* (allele 9/allele 1)	Loci *Xgwm512*/*SC-Y15* *R* (allele 2/allele 1)
Parents	0.99	0.79
PA1	0.86	0.64
PA5	0.82	0.60
PA10	0.88	0.64
PA17	0.60	0.36

At each locus, alleles have been numbered from 1 to *n* according to allele size (*n* the number of allele at the locus). The *ventricosa* alleles characteristic of the introgression were the allele 1 for *SC-Y15*, the allele 9 for *Xgwm636* and the allele 2 for *Xgwm512*

Detailed correlations between *ventricosa* alleles at *SC-Y15* and at markers *Xgwm636* and *Xgwm512* (Table 3) indicated very high correlations within the parents. The correlations decreased during inter-crosses and were then maintained from generation 1 to generation 10, whereas they decreased strongly between generations 10 and 17.

Evolution of multilocus associations between markers within the introgression

MSN networks of multilocus haplotypes for markers of the introgression *Xgwm636*, *SC-Y15* and *Xgwm512* were drawn at each generation (Fig. 5). Seven haplotypes were found within the parents. The four parents with the introgression (VPM, VM4, C10-7-3, C24-29) shared the same haplotype called *ventricosa*. In PA1, 16 new haplotypes appeared: two new haplotypes were due to the presence of new alleles at the *Xgwm636* microsatellite marker and the others can be explained by recombination. Numerous recombination events were observed between the three loci. Note that at least four double recombination were observed in PA1. These results showed that chromosome fragments near *Xgwm636* and *Xgwm512* in the lines bearing the introgression are homologous to those found in the lines without the introgression. Therefore, either the two loci were in the introgression and the alien fragment of *ventricosa* was inserted in the homoelogous region of wheat or they are wheat loci in the introgression. In PA5, five new haplotypes appeared, among which one was due to the presence of another new allele at the *Xgwm636* marker. *Ventricosa* haplotype frequency decreased between parents (0.25) and PA1 (0.18). In PA10, only one new haplotype appeared and the total number of haplotypes decreased concomitantly with selection of the *ventricosa* haplotype whose frequency reached 0.42. In PA17, new allelic associations appeared and *ventricosa* haplotype frequency was reduced from 0.42 to 0.23. Diversity calculated for the haplotype frequencies increased between the parents (0.85) and PA5 (0.90) then it decreased in PA10 (0.79) and increased again in PA17 (0.90).

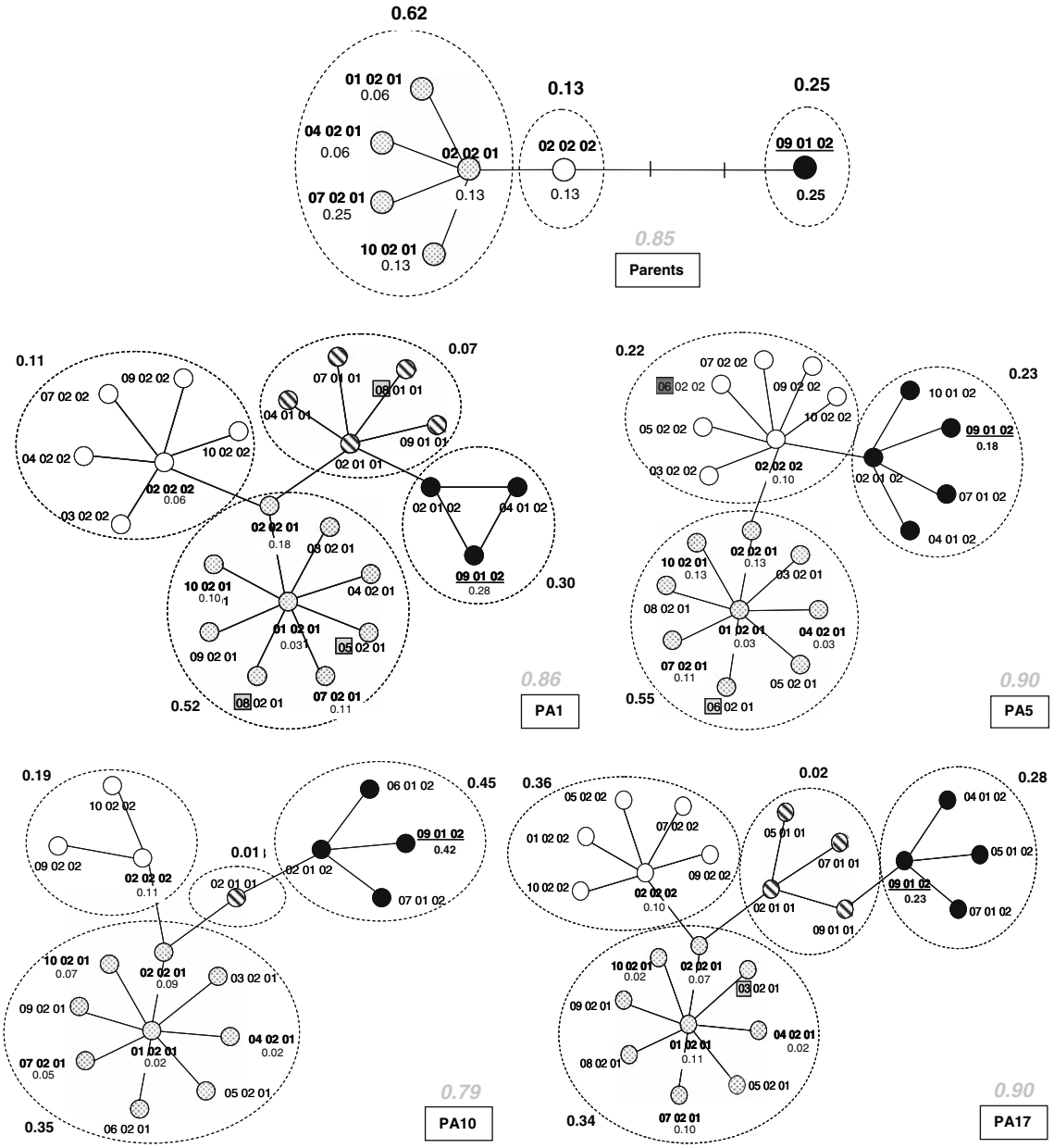

Fig. 5 Minimum Spanning Network drawn from haplotype "*Xgwm636-SC-Y15-Xgwm512*" at each generation. Haplotypes are represented by six numbers: the two first correspond to the *Xgwm636* allele, the two following correspond to *SC-Y15* allele and the two others correspond to the *Xgwm512* allele. Each group of haplotypes bring together haplotypes with the same allele to the locus *SC-Y15* and the same allele to the locus *Xgwm512*. Frequency of each group is indicated at each generation. Alleles in *gray square* indicate the appearance of a new allele at the locus *Xgwm636*. Parental haplotypes are represented in *bold letters* within each group with their frequency in the population. *Ventricosa* haplotype are *underlined* (**09 01 02**). At each generation, *gray letters* indicate the haplotype diversity calculated from haplotype frequency

Discussion

Selection of the *Yr17* resistance gene

The genetic effective population size estimated between parents and generation 17 from markers located on the whole genome was $N_{eg} = 167$. It was very low compared to the demographic effective size ($N_{ed} \approx 2650$) which was estimated based on the minimal true number of plants grown in the population (at least 5,000 plants per generation) corrected for the mean inbreeding coefficient ($\alpha \approx 0.9$; Goldringer et al. 2001). This suggested that the DM population was evolving at a faster rate than would be expected for a population

of 2,650 equivalent panmictic individuals under genetic drift only, indicating that other evolutionary forces such as selection could be involved. It is classical for N_{eg} to be lower than N_{ed}, as shown in a review of 192 experiments on natural populations by Frankham (1995), where the mean ratio was about 0.10. The first most important variables influencing the value of this ratio were the fluctuation in population size and variance in family size. Yet, as the number of individuals in the wheat experimental population is under control, we expected very few demographic fluctuations. Here, the low N_{eg} (=167) rather indicated that all plants did not contribute equally to subsequent generations, i.e. there was a large variance in reproductive contribution. This variance may be due to nonherited or inherited causes. In the latter case, selection would also increase the variance of reproductive contribution (by indirect effect) while modifying the frequency of the genes involved in the control of the fitness-related traits. In a previous study (Goldringer et al. 2001), using the analytical formulae (Caballero 1994) for the estimation of N_{eg} accounting for nonherited variation in the reproductive contribution, we found that only unrealistic variances could explain the discrepancy we observed between the estimated N_{eg} and the demographic size of the populations. On the other side, theory (Caballero, 1994) and some experimental results (Austerlitz and Heyer 1998) showed that low level of correlation between the effective family sizes at successive generations may strongly reduce genetic effective population size. This led us to conclude that differences in parental contributions were due to inherited genetic causes and that in other terms limited effective population size was due to selection.

It has been shown in previous studies that many traits (such as earliness, disease resistances, plant height) were submitted to diversifying or homogeneous selection in the DM population (Leboulc'h et al. 1994; Paillard et al. 2000a, b; Goldringer et al. 2001b, 2006). Selection might not only reduce the global effective population size (as described above), but it might also locally modify genetic diversity around the genes submitted to selection. This led us to investigate the evolution of genetic diversity around the *Yr17* specific resistance gene. The *Yr17* gene frequency, estimated from the frequency of a *SCAR* marker (*SC-Y15*), greatly varied across generations between parents and generation 17, with a significant increase between generations 5 and 10. Comparing the temporal variance of allelic frequencies to the global rate of evolution of the genome, we concluded that the gene had been under positive selection between these two generations and we estimated a coefficient of selection $s = 0.13$. This estimated value was high, suggesting a strong selection of the gene. Although different generations were studied here, it seemed difficult to use approaches based on the detection in selected progenies of segregation distorsion in allele frequencies at neutral markers such as the one proposed by Gomez-Raya et al. (2002). As suggested by Goddard (2003), such an approach does apply to the detection of selection in the wild only in rare situations because it requires studying offspring with large family size (>100) and their parent(s). In our case, it would need to develop appropriate procedures to account for the confounding of effects (unknown parentage relationship, genetic drift, mutation...) due to the bulk harvest of all individuals at each generation during several generations.

The observed evolution of *Yr17* frequency could be connected to the evolution of pathogenic pressures at Le Moulon across generations (Fig. 6). Indeed, a multisite and multiyear study on DM populations showed a significant effect of the presence of the *Yr17* gene on adult plant resistance to yellow rust (R^2 = 0.10–0.28; Paillard 1999). Epidemiologic data on pathogen pressures for an area including Le Moulon are available since 1975. Because of high sensibility of the pathogen to environmental conditions, the presence of rust diseases strongly changed among years (Hovmoller 2001). Strong rust attacks were observed in 1981, 1988, 1989, 1993 and 1999 whereas, the disease was absent between 1985 and 1987, in 1996 and in 1997 (de Vallavieille-Pope et al. 2000). Following yellow rust contamination, the disease develops very early and the pathogen can attack all parts of the plant with dehydration and grain shrivelling as a consequence. Yield losses up to 40% can be observed in cases of strong and early yellow rust attacks (Villaréal et al. 2002; Hovmoller 2001). Different yield components, number of spikes per plant, number of kernels per spike and mean kernel weight can be affected. Seeds matured on highly infected plants germinate poorly and irregularly (Paillard 1999). Hence, plants with the *Yr17* specific resistance gene would have a selective advantage over the others under strong yellow rust epidemic with race strains to which they would be resistant. This would lead to an increase in the resistance gene frequency in the population. The increase of *Yr17* gene frequency between parents and generation 1 can be explained by a strong disease attack in 1981. Between generations 1 and 5, the disease was absent and we can consider that selection was relaxed. Between generations 5 and 10, the resistance gene increased significantly concomitantly with strong yellow rust

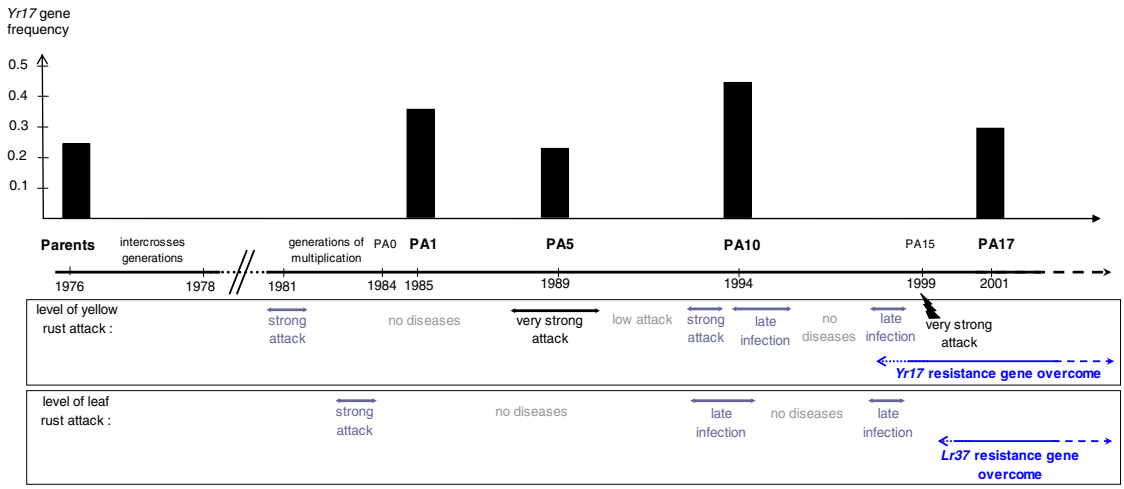

Fig. 6 Evolution of the *Yr17* gene frequency correlated to the level of yellow and leaf rusts attacks during generations

attacks in 1988, 1989 and 1993. Between generations 10 and 17, the gene frequency decreased despite a very strong attack in 1999. This result may be explained by the emergence of a new race of the pathogen to which *Yr17* was not resistant. This new virulent race was observed for the first time in 1994 in the UK and Denmark and in 1997 in France (Bayles et al. 2000). The specific virulence to *Yr17* was observed at Le Moulon in 1998 (Paillard 1999). During the very strong epidemic of yellow rust in 1999, most individuals carrying the *Yr17* gene became susceptible, whereas individuals with other sources of partial resistance were possibly favored within the population. When selection of the gene was relaxed, between generations 1 and 5 and after the overcome of the resistance gene by the pathogen, we observed a decrease in the gene frequency associated with a particularly low effective size. This suggested that the *ventricosa* introgression could be associated with some negative effects within the background of cultivated wheat. In a review, Brown (2002) related several cases of introgression of resistance genes from wild species linked to yield depression. Helguera et al. (2003) suggested using isogenic lines carrying the resistance gene to quantify the effect of this introgression on agronomical quality. But so far, the fitness cost for genotypes carrying the *ventricosa* chromosome fragment has not been estimated. Moreover, Hovmoller (2001) showed that the variety ''Brigadier'' completely resistant to the yellow rust before 1995 thanks to the presence of the *Yr17* gene was twice more affected by the pathogen than the susceptible control in 1997 when the new virulent race appeared. Such a strong increase in susceptibility for genotypes carrying the *Yr17* gene after the overcome of the resistance gene could explain the rapid decrease of *Yr17* gene frequency in the population between generations 10 and 17.

Because the *Yr17* resistance gene is tightly linked to *Lr37* and *Sr38*, two resistance genes to leaf rust and steam rust located in the same introgression, changes in pathogen pressures for the two other diseases also need to be considered when explaining the evolution of the chromosomal zone. Steam rust disease was never observed at Le Moulon since the beginning of the DM program in 1984. As for leaf rust, this disease is mostly observed in the south of France. Some rare attacks occurred at Le Moulon which were associated with yellow rust attacks and could thus have increased the effect of selection on the chromosome zone between parents and PA1 and between PA5 and PA10. The *Lr37* resistance gene was overcome in 2000 in France by the appearance of a pathogen race to which it was not resistant (H. Goyeau, personal communication), and individuals carrying this resistance gene lost any selective advantage.

Impact of selection on diversity and on linkage disequilibrium near the Yr17 resistance gene

Genetic effective population size calculated for each locus showed that the markers close to the gene (*Xgwm636*, *Xgwm512* and *Xgwm359*) were affected by the strong selection of the gene between generations 5 and 10. Conversely, markers distant from the gene were not affected by the selection of *Yr17* and allelic frequencies at these markers evolved randomly under the effect of genetic drift. So, *Yr17* strong selection induced indirect selection of neutral markers over a distance of about 20 cM.

Selection is expected to reduce diversity at linked loci through the hitch-hiking effect. In the case of *Yr17* selection, there was no evidence for a loss of diversity at the mono-locus level in the region surrounding the selected gene. The *Xgwm636* marker located 5 cM away from the selected gene was the only one to show a decrease of diversity correlated with selection. Moreover, allelic richness of the chromosomal zone surrounding the gene was not affected by selection. The short duration of the selection combined with the low initial frequency of the favorable allele may explain the limited impact of *Yr17* gene selection observed on genetic diversity. Reduction of neutral variation around a selected gene depends on the initial frequency of the selected gene (Innan and Kim 2004), and on the intensity of selection. In our case, selection led to an increase in allele frequency up to 0.5 inducing an increase of gene diversity up to its maximal value for a biallelic locus during selection contrary to what is expected in a case of fixation of the selected gene. Although a decrease of diversity within individuals possessing the resistance gene was observed between generations 5 and 10 compared to individuals without the gene, at the population level the estimation of genetic effective population sizes at the different loci close to the gene under selection was more powerful for detecting a signature of selection than was the monolocus estimator of genetic diversity. We think that this was due to the short selection duration. In addition, the approach of Goldringer and Bataillon (2004) based on the estimation of temporal variance in allele frequencies was more robust to new alleles than would be approaches based on monolocus gene diversity or LD. Whereas it is not likely that mutation (that yields new alleles at very low frequencies) influences the evolution of allele frequencies over time except in case of recurrent mutation over a large number of generations, new alleles at low frequencies would increase gene diversity as well as they would randomly modify LD. Therefore, looking for the signature of selection using variation in allele frequencies seemed more appropriate for microsatellite diversity survey whereas the latter two approaches seemed more useful when using SNPs.

Population genetics theory predicts that selection will increase LD between a selected locus and neutral linked markers and between neutral linked markers themselves. In this study, a small increase of LD was observed between markers flanking the resistance gene within a 20 cM region and the resistance gene itself correlated with selection. LD is largely governed by recombination and decays with genetic distance. Due to the presence of an alien genome fragment restricting recombination, the population showed strong LD between markers of the introgression and the *Yr17* resistance gene within a region of about 15 cM until generation 10. Selection contributed to the reinforcement of the pre-existing LD in the zone surrounding the resistance gene between generations 5 and 10.

However, studying the evolution of allelic associations within the introgression markers has enabled highlighting numerous losses of association between *ventricosa* alleles due to multiple recombination before generation 1, favored by the four consecutive generations of inter-crosses. Our results provided evidence that some recombination events occurred between *T. aestivum* 2AS and *A. ventricosa* 2NS chromosome segments. Bariana and McIntosh (1993) estimated a recombination rate of 1% between the two resistance gene *Yr17* and *Lr37*. Robert et al. (2000b) found that 2% of their lines had conflicting results between the expected presence of the resistance genes on the basis of their *SCAR* marker for the 2NS segment and the resistance tests for *Yr17*, suggesting the possibility of a certain level of recombination within the introgression between the marker and the resistance gene. The presence of an alien introgression in some individuals does not fully suppress recombination as it is the case for some other wheat alien introgression, but recombination tends to be reduced as shown by the strong correlation between *ventricosa* alleles until generation 10.

Whereas the evolution across generations of monolocus diversity did not indicate any local loss of diversity due to selection, the multilocus diversity assessed within the introgression showed a clear decrease correlated with the selection of the *Yr17* gene. The number of haplotypes increased at all generations due to the appearance of new haplotypes either by recombination or emergence of new alleles except between generations 5 and 10 where selection tended to reduce the number of haplotypes in favor of the resistant *ventricosa* haplotype selected. In a previous study, we analyzed the evolution over time in the same population of microsatellite diversity at the same 21 loci distributed all over the genome focusing on the emergence of new alleles (Raquin 2005; A.L. Raquin et al., submitted). Investigating the origin of these new alleles, we found that the independent emergence of new alleles, the polymorphism in the number of repeat units and the absence of evident contamination by external pollen or seed allowed us to conclude that the new alleles observed in the population appeared by mutation. From this, we assumed that the new haplotypes due to presence of new alleles at the *Xgwm636* loci most probably have appeared by mutation.

What is known about map distance and ordering of the markers?

In this study, the markers were mapped using public mapping data available for the ITMI population; it allowed us to localize the relative position of the microsatellite markers. The parents of this mapping population carried no introgression and no precise map of the *Yr17* gene has been published to date to our knowledge. *SC-Y15* was approximately located combining data from F. Dedryver (personal communication) and mapping data but its position relative to *Xgwm512* and *Xgwm636* is still uncertain. However, LD has been maintained across generations between *SC-Y15* and these two markers at a higher level than LD with other markers. This was consistent with the hypothesis that the two markers were closer to the gene than any other. The delimitation of the *ventricosa* introgression is still inaccurate in the literature: Helguera et al. (2003) mapped the introgression from RFLP markers and found a length between 25 and 38 cM representing approximately half of the short arm of the chromosome 2A. Although the mapping position of *Xgwm636* was uncertain in the literature, in our study strong LD estimated between *Xgwm512* and *Xgwm636* and *SC-Y15* suggested that the two microsatellite markers belong to the introgression. Our results also suggested that the *Xgwm359* marker could belong to the introgression. Indeed, the four parents having the introgression presented the same allele at this marker and we found a strong LD between this allele and the ''*ventricosa*'' allele of the *SC-Y15*. This strong LD was maintained until generation 5 and increased at generation 10 up to 0.42, probably as a result of selection, as for the other markers of the introgression, whereas this trend was not observed for *Xwmc177*.

To our knowledge, this study is the first to examine the impact of selection in a population with a mainly selfing mating system and considering the specific case of recombination restriction. In another study on the same population, A.L. Raquin et al. (submitted) showed that the selection on *Rht-B1*, the major dwarfing gene through the 24 generations of evolution between the parents and PA17 had almost no effects on diversity and LD in the region around the gene. The frequency of the ''tall'' allele increased regularly across generations until quasi-fixation. This strong selection was associated to a loss of gene diversity similar to what was observed overall the genome. Moreover, the LD observed between the markers close to the selected gene became rapidly nonsignificant across generations. The impact of selection on the region (6 cM) flanking the *Rht-B1* gene was very limited compared to the impact observed on a region of 20 cM for the *Yr17* selection indicating that the selective sweep around the *Yr17* resistance gene was specifically due to its location in a 15–20 cM alien introgression presenting restriction to recombination. Reversely, in the long term program of experimental barley populations, Danquah and Barrett (2002) showed a significant evolution in hordein pattern within the population introduced in Cambridge, UK. They interpreted this evolution as the signature of selection of the powdery mildew resistance gene *Mla1*, which is closely flanked by two hordein genes *Hor1* and *Hor2* separated by 7 cM from each other. The authors assumed that selection on the mildew resistance gene in relation with disease selection pressure led to a significant change at the two hordein loci through a hitch-hiking effect. How selection will influence the organization of diversity depends, in particular, on the recombination rate and on the mating system. The effects of selection have been often studied in outcrossing species. For instance, in maize, Clark et al. 2004 studied the evolution near the domestication *tb1* gene which controls the increase in apical dominance in maize relative to teosinte and showed an impact of selection limited to approximately 60 kb due in particular to the high effective recombination rate. In the malaria parasite *Plasmodium falciparum*, Nair et al. (2003) observed reduced variation for approximately 100 kb (6 cM) around the selected *dhfr* gene during rapid selection of the resistance gene to antimalarial drugs. However, a hitch-hiking effect on a region up to 32 cM was found by Kohn et al. (2000) in a natural rat population submitted to selection for resistance to anticoagulants.

Implications for dynamic conservation

Dynamic management populations are submitted to constant natural selection affecting the whole genome. The diversity of selective pressures within the DM network has enabled the populations cultivated in different environments to maintain polymorphism on loci involved in adaptation. In addition, the relatively high differentiation among populations for neutral genetic diversity ($F_{st} \approx 0.04$–0.11 after ten generations, Enjalbert et al. 1999), the conservation of allelic richness over the different populations (Goldringer et al. 2001b), the residual outcrossing (Enjalbert et al. 1998) and the observed mutation events indicated that neutral diversity was maintained at the whole genome level due to the different evolutionary pressures aside from migration which up to now has not been applied between the DM population. One of the objectives of

this study was to assess whether selection within a given population could strongly reduce genetic diversity around selected genes through hitch-hiking effects, inducing a need for a specific renewing of local diversity. Studying the evolution of diversity within the region flanking a major selected gene (*Rht-B1*, A.L. Raquin et al., submitted) in a DM selfing population showed a very limited impact of selection when recombination was occurring at the regular rate. Impacts of selection for the flanking regions of the *Yr17* gene were greater, probably because it is located in an introgression. However, the selection of *Yr17* resistance gene appeared to be too limited in time to significantly affect allelic richness of markers near the gene. In addition, the initial multiplecross and a significant mean outcrossing rate allowed recombination to occur within the introgression and thereby limited the impact of selection. This higher than expected level of recombination within the introgressed fragment showed the usefulness of such long term management program to help incorporate gene from the wild pool into cultivated varieties. The loss of efficiency of the *Yr17* resistance gene by the emergence of a pathogen race to which it was not resistant between generations 10 and 17 stopped the selection of the gene. A rapid return to equilibrium was observed with a decrease of the LD between markers around the gene. These results showed the ability for rapid evolutions of this wheat population which is compatible with the objectives of the DM program.

Acknowledgments The authors thank N. Galic for technical assistance, G. Boutet for the genotyping of the population PA17, F. Dedryver for personal data on the *SCAR* and R. Bernardo, I. Bonnin, P. Brabant and J. Shykoff for careful reading of the manuscript. This work was supported by a grant from the ''Bureau des Ressources Génétiques''.

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