Breakdown of the Scab Resistance Gene *Vf* in Apple Leads to a Founder Effect in Populations of the Fungal Pathogen *Venturia inaequalis*

Fabien Guérin and Bruno Le Cam

UMR Pathologie Végétale, INRA, INH, Université d'Angers, Centre INRA d'Angers, B.P. 57, 42 rue Georges Morel, 49071 Beaucouze, Cedex France.

Accepted for publication 15 December 2003.

ABSTRACT

Guérin, F., and Le Cam, B. 2004. Breakdown of the scab resistance gene *Vf* in apple leads to a founder effect in populations of the fungal pathogen *Venturia inaequalis*. *Phytopathology* 94:364-369.

The recent breakdown of *Vf*, a major resistance gene to apple scab, provided an opportunity to analyze a population genetic process within the matching virulent subpopulation of the fungus *Venturia inaequalis*. We utilized the amplified fragment length polymorphism technique and allelic variation at four microsatellite loci to assess genetic structure of 133 isolates of *V. inaequalis* from a single commercial apple orchard sampled from one cultivar carrying the *Vf* gene (Judeline) and three cultivars devoid of the *Vf* gene. Both analyses indicated a strong decrease of

the genetic diversity among isolates from the *Vf* cultivar compared with the high level of diversity among isolates from the three other cultivars. This leads to a high genetic differentiation between *virVf* and *avrVf* groups ($F_{ST} > 0.17$). Analyses of the genetic distance between AFLP patterns based on the Jaccard index indicate that all *virVf* isolates could be assigned to a single clonal lineage. These results lead us to conclude that the clonal structure of the population isolated from the *Vf* cultivar is an example of a founder effect in response to a resistance gene breakdown and it is likely that this event occurred in the orchard during the sampling year.

Additional keywords: gene-for-gene relationship, simple sequence repeat.

Fungal pathogen populations of agricultural crops are under constant selection pressure to increase their ability to infect new resistant host cultivars implemented by growers. It is crucial to know the process by which fungal populations adapt to host resistance to improve management, breeding, and introduction of new resistant cultivars toward a sustainable agriculture (5,19). The genetic basis of host resistance is based on both quantitative trait loci, responsible for partial resistance, and on race-specific resistance (major gene). In the latter case, one resistance gene interacts with one avirulence gene (*avr*), leading to the gene-for-gene relationship (12). In this type of interaction, introduction of a resistance gene first leads to a reduction or even a local extinction of the pathogen population on plants carrying the gene if the matching virulence (*vir*) is not present in the ecosystem. Subsequently, molecular events within the functional avirulence allele, such as a single mutation, may lead to production of isolates able to overcome the resistance gene (14). Ability of virulent isolates to adapt more or less rapidly to plants carrying race-specific resistance has been reviewed or modeled in both natural ecosystems and agricultural systems (5,8,38). For example, powdery mildew disease control under such a monogenic resistance lasted 2 to 5 years in both wheat and barley crop in Europe before it was overcome by a few virulent clones (6).

Venturia inaequalis (Cooke) G. Wint. is responsible for scab, the major apple disease in most areas of the world. It is a haploid filamentous ascomycete that fits the scheme of gene-for-gene interaction with its host *Malus × domestica* (1,3). This pathogen's life cycle is based on one sexual cycle occurring each year followed by many vegetative cycles leading to a high genetic diver-

sity within populations (35). It is likely that such a reproductive system will facilitate fungi to adapt to specific resistance genes of the host by both increasing risks of avirulence losses and spreading of new virulences. By 1969, five distinct scab resistance genes had been identified in wild apple germ plasm (43); a sixth gene named *Vr* from apple cv. Antonovka was reported in 1989 (21). Among the six genes, only the resistance gene *Vf* from *Malus floribunda* clone 821 has been widely introgressed in improved apple genotypes released by plant breeders. Despite the scarce use of cultivars harboring this gene in commercial orchards to date, it has been rapidly overcome in several European countries (22,27,28); observation of scabbed leaves also was reported in a single year on cv. Baujade (*Vf*) in New Zealand (9) with no further extension. In France, the first breakdown of the *Vf* gene was reported in a single orchard of cider apples in 1995 on cv. Judeline (26), with no further reported extension from this orchard. During the year 2000, strong apple scab symptoms were observed on *Vf* cv. Judeline in a commercial cider orchard in Normandy (France) 100 km from the site of the previously reported first *Vf* breakdown. The heavy apple scab infection observed in this orchard allowed us to analyze a population genetic event linked to the occurrence of virulence matching the *Vf* resistance gene.

Since in a gene-for-gene interaction, a mutational event occurring within a single individual is assumed to lead to the selection of one new population responsible for an epidemic disease, we aimed to test the existence of a founder effect within populations of *V. inaequalis* subsequent to the breakdown of a major resistance gene. Four sets of isolates from the same orchard were analyzed and compared: one set was isolated from cv. Judeline, which carries the *Vf* gene (*Vf* cultivar), and the others were from three cultivars devoid of the *Vf* gene (non-*Vf* cultivars). To assess population genetic structure, microsatellite loci (simple sequence repeat [SSR]) are widely used in many areas of genetics because of the high variability in the number of repeat units (7,33). The

Corresponding author: F. Guérin; E-mail address: fguerin@angers.inra.fr

Publication no. P-2004-0120-02R

© 2004 The American Phytopathological Society

amplified fragment length polymorphism (AFLP) technique is also a rapid and reliable method successfully used to investigate intra-specific variations within fungal species (15,24,39). A genetic map under construction in our laboratory indicates that these markers span the genome of *V. inaequalis* and therefore provide information over many independent loci. By utilizing both the AFLP technique (40) and the allelic variations at four microsatellite loci (36), a clear picture of the population structure should emerge.

MATERIALS AND METHODS

Fungal materials and DNA extraction. The fungal isolates used in this study originated from a single orchard containing several cultivars, including the *Vf* cultivar (Judeline) and non-*Vf* cultivars (Petit Jaune, Juliana, and Judor), in Pont-Audemer (49.35°N; 8.52°E), Northwest France. Isolates of *V. inaequalis* were collected on 19 September 2000 from trees chosen at random. Strains and cultivars of origin are listed in Table 1. Strains were derived from monoconidial isolates and stored on malt agar (Merck, Darmstadt, Germany) at 4°C. Procedures of DNA extraction from fungal culture were described previously (20). Other DNA samples were directly extracted from leaf lesions exhibiting scab sporulation (18).

Microsatellite and AFLP markers. All of the strains were screened with four nuclear microsatellite loci developed from *V. inaequalis*, named *Itc1a*, *Itc1b*, *Itc1g*, and *Iaac3b* (36), and with one AFLP primer-enzyme combination. Microsatellite amplifications were performed in a programmable thermocycler (geneAmp PCR System 9700; Applied Biosystems, Foster City, CA) as described by Tenzer et al. (36), except for the locus *Itc1g* amplification in which annealing temperature was decreased to 50°C instead of 58°C. Microsatellite markers were labeled with non-radioactive fluorescent dyes.

All of the strains derived from monoconidial isolation were submitted to fluorescent AFLP (*fAFLP*) analysis. A first reaction mixture consisted of 3.6 units each of *Pst*I and *Msp*I (Eurogentec SA, Herstal, Belgium), 2 µl of 0.5 M NaCl, 0.2 µl of 100× bovine serum albumin (New England Biolabs, Beverly, MA), 1.5 µl of 10× T4 ligase buffer (New England Biolabs), 1 µl of 2 µM *Pst*I adapter, and 1 µl of 20 µM *Msp*I adapter (Table 2) in a final volume of 10 µl. A second reaction mixture consisted of 0.5 µl of 10× T4 ligase buffer and 0.2 µl of T4 DNA ligase (New England Biolabs). One hundred fifty nanograms of genomic DNA (5 µl) was mixed with the first and the second mixture and then incubated for 3 h at 37°C. After incubation, 20 µl of the reaction mixture was diluted up to 200 µl with water. Two amplifications—preselective and selective—were then performed. The preselective amplification was performed in 20 µl containing 4 µl of the diluted DNA resulting from the restriction-ligation step, 2 µl of 10× Goldstar buffer (Eurogentec SA), 5 mM MgCl₂, 2 mM dNTPs (Eurogentec SA), 0.25 µM *Pst*I primer and *Msp*I primer (Table 2), and 0.5 units of *Taq* DNA polymerase (Goldstar Red; Eurogentec SA). It consisted of incubation at 72°C for 2 min followed by 20 cycles of the following profile: a 20-s DNA denaturation step at 94°C, a 30-s annealing step at 56°C, and a 2-min extension step at 72°C. The polymerase chain reaction (PCR) products were then diluted up to 200 µl with water. The selective amplification using the 5'-FAM-labeled *Pst*I+T primer (Table 2) and the unlabeled selective *Msp*I+TT primer was performed in a 20-µl reaction containing 1.5 µl of diluted preamplified DNA, 2 µl

of 10× Goldstar buffer, 2.5 mM MgCl₂, 2 mM dNTPs, 50 nM *Pst*I+T primer, 250 nM *Msp*I+TT primer, and 0.5 units of *Taq* DNA polymerase. It consisted of a 2-min denaturation step at 94°C followed by 33 cycles: 94°C for 20 s, annealing step for 30 s, and 72°C for 2 min. The annealing temperature was 66°C for the first cycle and then decreased by 1°C increments for the next nine cycles and was 56°C for the last 23 cycles. A final extension step was performed at 60°C for 30 min. All of the amplification reactions were performed in a programmable thermocycler. At least two independent *fAFLP* replicates were performed in order to retain only peaks detected in all replicates.

Both microsatellite and *fAFLP* amplification products were detected using an automatic sequencer (ABI 310) and analyzed with GENESCAN 3.1 software (Applied Biosystems). The inclusion of size standards labeled with a different fluorescent dye (400HD-ROX for microsatellites analysis and 500-TAMRA for *fAFLP* analysis) enabled samples to be aligned using GENESCAN 3.1 software.

Data analysis of microsatellite markers. DNA of *V. inaequalis* strains was analyzed from haploid mycelia. Allele frequencies, variance in allele size (V_{sz}), and number of alleles (A) at each locus were analyzed for all isolates. A standard gene diversity index (25), which is equivalent to the expected heterozygosity for diploid data, was calculated. This index is defined as the probability that two randomly chosen haplotypes are different. Gene diversity is estimated as $H_d = [n/(n-1)](1 - \sum_{i=1}^k p_i^2)$, where n is the number of gene copies in the sample, k is the number of haplotypes, and p_i is the sample frequency of the i th haplotype. Assessment of genetic structure was performed using the infinite alleles model (17). Values of F_{ST} (42) were calculated using the program ARLEQUIN, version 2.00 (31), in which significance levels for the overall values and for the population pairwise values were determined after 3,024 permutations.

Data analysis of *fAFLP* markers. *fAFLP* bands of 70 to 500 bp were scored. Only DNA extracted from fungal cultures derived from monoconidial isolates was analyzed. *fAFLP* bands that were present in some isolates and absent in other isolates were considered polymorphic. Sample files were imported into GENOTYPER version 2.0 (Applied Biosystems), and all samples were normalized. Initially, potential markers were generated by the software, and fragments with clearly separated size ranges and overall high signals (fragments with scaled peak heights mainly above 200 units), that could be scored unambiguously for all samples were selected manually. The presence and absence of a polymorphic marker was coded with 1 and 0, respectively, resulting in a binary data matrix of the different *fAFLP* multilocus phenotypes. For all populations, amount of polymorphism and average gene diversity over loci were determined. Assessment of genetic structure was performed as described previously.

TABLE 2. Adaptors and primers used for fluorescent amplified fragment length polymorphism analysis

Adapter	Primers
<i>Pst</i> I adapter	5'-TCGTAGACTGAGTACGTGCA-3' 3'-CATCTGACTCATGC-phosphorylation-5'
<i>Pst</i> I primer + T	5'-6-FAM-GACTGAGTACGTGCAGT-3'
<i>Msp</i> I adapter	5'-GACGATGAGTCCTGAG-3' 3'-CTACTCAGACTCGC-phosphorylation-5'
Selective <i>Msp</i> I primer + TT	5'-CGATGAGTCCTGAGCGGT-3'

TABLE 1. Isolates of *Venturia inaequalis* used in this study

Strain	Location and date of isolation	Cultivar
P-1 to P-36	France (49.35°N, 8.52°E) – 2000	<i>Malus × domestica</i> cv. Judeline (<i>Vf</i>)
PJ-1 to PJ-32	France (49.35°N, 8.52°E) – 2000	<i>Malus × domestica</i> cv. Petit Jaune
JLN-1 to JLN-30	France (49.35°N, 8.52°E) – 2000	<i>Malus × domestica</i> cv. Juliana
JDR-1 to JDR-35	France (49.35°N, 8.52°E) – 2000	<i>Malus × domestica</i> cv. Judor

Pairwise distances. Pairwise distances between all isolates were calculated from the binary *f*AFLP data matrix using the Jaccard coefficient in the software DISTAFLP (37). The binary data set was subjected to bootstrap analysis with 100 replications using the same program. A phenogram based on the resulting distance matrix was constructed using the neighbor-joining method (30) as implemented in the program NEIGHBOR from the PHYLIP package (available online from the University of Washington, Department of Genetics) and drawn with NJPLOT (29).

TABLE 3. Allele frequencies and unbiased gene diversities (H_d) of *Venturia inaequalis* populations virulent on *Vf* and non-*Vf* cultivars obtained with the four microsatellite markers

Locus	Allele (bp)	Judeline (<i>Vf</i>) ($n = 36$)	Petit Jaune (non- <i>Vf</i>) ($n = 32$)	Juliana (non- <i>Vf</i>) ($n = 30$)	Judor (non- <i>Vf</i>) ($n = 35$)
<i>Itc1a</i>	107	...	0.0625	0.2667	0.0857
	112	...	0.0938	...	...
	120	...	...	...	0.0286
	122	...	...	0.0333	0.0571
	124	...	0.0313	0.1333	0.0571
	126	1.000	0.5625	0.3000	0.5143
	128	...	0.1875	0.1667	0.0857
	132	...	0.0625	0.0333	...
	134	...	...	...	0.0571
	136	...	...	...	0.0857
	146	...	...	...	0.0286
	154	...	...	0.0667	...
	H_d	0.000	0.6409	0.8000	0.7122
<i>Itc1b</i>	144	...	...	0.0333	...
	148	...	...	0.3000	0.0571
	158	...	...	0.0333	0.0571
	166	...	...	0.1000	...
	168	1.000	0.5938	0.2667	0.4000
	170	...	0.4063	0.2000	0.4857
	192	...	...	0.0667	...
<i>Itc1c</i>	H_d	0.000	0.4901	0.7955	0.6062
	115	1.000	0.8125	0.7586	0.7429
	118	...	0.0625	0.2414	0.0286
	121	...	0.1250	...	0.2286
<i>Itc1d</i>	H_d	0.000	0.3254	0.3727	0.4008
	117	...	...	0.0333	...
<i>Itc1g</i>	127	...	0.0313	...	0.0857
	129	...	0.0313	...	...
	133	...	...	0.1000	0.2000
	135	...	0.2500	...	0.0571
	137	...	...	0.0667	...
	139	...	0.0313	0.0333	...
	141	...	0.0625	0.1000	0.1143
	145	...	...	0.0667	0.0571
	147	0.8889	...	0.0333	...
	149	0.1111	...	...	...
	153	...	0.2813	0.1000	0.0571
	155	...	0.0625	0.1333	0.0857
	157	...	0.0625	...	...
	159	...	...	0.1000	0.0857
	161	...	0.0625	...	0.0286
	163	...	...	...	0.1714
	165	...	0.0313	0.1000	...
	167	...	...	...	0.0286
	168	...	...	0.0333	...
	173	...	0.0313	0.0333	...
	175	...	...	0.0667	...
<i>Itc1h</i>	183	...	...	...	0.0286
	187	...	0.0625	...	...
H_d		0.2003	0.8472	0.9288	0.8961
Number of alleles per locus ^a		1.25	5.75	7.5	7
H_d averaged ^a		0.0500	0.5759	0.7242	0.6538
Number of haplotypes		2	20	23	25

^a Values are means.

RESULTS

Microsatellite analysis: Allele frequency distributions and gene diversities within populations. Allele frequencies and gene diversities are given for each population in Table 3. The total number of alleles detected in all samples ranged from 3 (locus *laac3b*) to 24 (locus *Itc1g*). The mean number of alleles per locus per cultivar group ranged from 1.25 (*Vf* cv. Judeline) to 7.5 (non-*Vf* cv. Juliana). Among isolates virulent on the *Vf* cv. Judeline, only one allele was revealed at each locus, except for locus *Itc1g* where two close alleles were found (147 and 149 bp). These results lead to a low number of different haplotypes isolated from the *Vf* cv. Judeline, where only two close haplotypes were revealed. Conversely, in the isolates from non-*Vf* cultivars (Petit Jaune, Juliana, and Judor), several alleles were characterized at each locus, revealing a total of 66 different haplotypes. Averaged gene diversities ranged from 0.05 for the isolates from the *Vf* cv. Judeline and from 0.5759 to 0.7242 for those isolated from non-*Vf* cultivars. The subpopulation virulent on the *Vf* cultivar (vir Vf) Judeline had significantly lower average gene diversity than the three other subpopulations at the 5% level (Mann Whitney's test, $P = 0.032$) with the null hypothesis H_0 : H_d value from population virulent on *Vf* cv. Judeline is not lower than H_d values from other cultivars.

Genetic structure. To assess the distribution of the genetic variation within and among populations, the following hypotheses concerning the allelic and genotypic distribution across all subpopulations and between subpopulation pairs were tested. For each locus, a probability test was performed on contingency tables and an estimate of P was calculated. The first null hypothesis tested was "the allelic distribution is identical across populations." P values were highly significant ($P < 0.05$), indicating the presence of a genetic structure. The second null hypothesis tested was "the genotypic distribution is identical across subpopulations." P values were again highly significant ($P < 0.05$), indicating the presence of a genetic structure. In order to measure the degree of population differentiation, we calculated F_{ST} between population pairs. All of the F_{ST} values for pairwise comparisons between *Vf* and non-*Vf* subpopulations were highly significant at the 5% level (Table 4). These results indicated a strong genetic differentiation between the population of *V. inaequalis* isolated from the *Vf* cv. Judeline and populations that originated from the three non-*Vf* cultivars. In contrast, no genetic differentiation was detected among the populations isolated from the non-*Vf* cultivars.

***f*AFLP analysis.** We exclusively analyzed DNA extracted from fungal cultures with the *f*AFLP technique because of the non-specificity of this PCR-based technique. Finally, 72 isolates of the 133 collected were analyzed (17 from Judeline, 20 from Petit Jaune, 21 from Juliana, and 14 from Judor). A total of 71 distinct and reproducible *f*AFLP fragments were identified with the combination of primers *Pst*+*T/Msp*+*TT*. Thirteen of the fragments were monomorphic for all isolates. As a whole, 60 unique *f*AFLP multilocus haplotypes were distinguished: five different haplotypes were characterized among the strains isolated from the *Vf* cv. Judeline, with one haplotype representing 59% of the popu-

TABLE 4. Matrix of pairwise comparisons of population genetic differentiation (F_{ST}) calculated using the four microsatellite loci^a

Host cultivar	Judeline (<i>Vf</i>) ($n = 36$)	Petit Jaune (non- <i>Vf</i>) ($n = 32$)	Juliana (non- <i>Vf</i>) ($n = 30$)	Judor (non- <i>Vf</i>) ($n = 35$)
Judeline	—	—	—	—
Petit Jaune	0.4262	—	—	—
Juliana	0.4221	0.0220*	—	—
Judor	0.4110	0.0263*	0.0170*	—

^a * Indicates not significant at 5% level.

lation, and average gene diversity over loci within this population was 0.0408 (Table 5). In contrast, all of the strains isolated from the three non-*Vf* cultivars showed a high level of haplotypic diversity (100%), and the average gene diversity ranged from 0.1984 to 0.2124 (Table 5). F_{ST} values for pairwise comparisons between the population isolated from the *Vf* cv. Judeline and populations isolated from the three other non-*Vf* cultivars were all significant at the 5% level ($0.1714 \leq F_{ST} \leq 0.1816$) and no genetic differentiation was revealed ($F_{ST} = 0$) for pairwise comparisons between populations isolated from non-*Vf* cultivars (Table 6). The neighbor-joining tree showed one clade clustering all of the strains isolated from the *Vf* cv. Judeline, which is by bootstrapping significantly distinct from the other clade (Fig. 1).

DISCUSSION

The objective of this study was to describe the structure of a *V. inaequalis* population in relation to host cultivars in an orchard where the *Vf* resistance gene was overcome. Contrary to the *avrVf* isolates that displayed a high level of polymorphism, the *virVf* isolates displayed a clonal one, which suggest the existence of a founder effect linked to the loss of an avirulence trait within a fungal population fitting the gene-for-gene model with its host. Genetic structure analysis provides evidence that the breakdown event occurred during the sampling year in this orchard.

High diversity and lack of genetic structure within the *avrVf* populations. Our first concern was to ascertain the existence of genetic diversity in the orchard to provide evidence of a population genetic event. Previous studies assessing the genetic diversity in this species at the European scale clearly indicated a high level of polymorphism of this fungus and a complete lack of genetic structure between countries and within orchards (34–36). Using four SSR loci, we showed that three subpopulations of *V. inaequalis* isolated from non-*Vf* cultivars (Petit Jaune, Juliana, and Judor) displayed a very high genetic diversity ($0.5759 \leq H_d \leq 0.7242$) and no significant differentiation ($F_{ST} = 0$). Similar results were revealed in the *f*AFLP analyses. Our data underscores the major role played by gene flow, which is in accordance with the biology of the fungus that combines an obligatory sexual stage each year and several vegetative cycles during the epidemic phase. The genetic diversity found in field populations of *V. inaequalis* was comparable to that found in *Mycosphaerella graminicola* (10) and *Phaeosphaeria nodorum* (16), which are also characterized by a high degree of sexual reproduction. The random distribution of alleles among populations of pathogens seems to suggest a lack of selective pressure from the host. Given the lack of

TABLE 5. Average gene diversity (H_d) over loci (71 loci), number of polymorphic sites, and number of haplotypes obtained with fluorescent amplified fragment length polymorphism analysis

	Judeline (<i>Vf</i>) (<i>n</i> = 17)	Petit Jaune (non- <i>Vf</i>) (<i>n</i> = 20)	Juliana (non- <i>Vf</i>) (<i>n</i> = 21)	Judor (non- <i>Vf</i>) (<i>n</i> = 14)
H_d	0.0408	0.2124	0.1984	0.2078
Polymorphic sites	9	52	46	43
Haplotypes	5	20	21	14

TABLE 6. Matrix of pairwise comparisons of population genetic differentiation (F_{ST}) calculated using the fluorescent amplified fragment length polymorphism loci

Host cultivar	Judeline (<i>Vf</i>) (<i>n</i> = 17)	Petit Jaune (non- <i>Vf</i>) (<i>n</i> = 20)	Juliana (non- <i>Vf</i>) (<i>n</i> = 21)	Judor (non- <i>Vf</i>) (<i>n</i> = 14)
Judeline	—	—	—	—
Petit Jaune	0.1725	—	—	—
Juliana	0.1714	0.0000	—	—
Judor	0.1816	0.0000	0.0000	—

correlation between AFLP patterns and pathotypes reported elsewhere (32,39), we cannot determine the effect of selection without data based on selected markers. High diversity in the orchard as well as evidenced by appropriate genetic markers such as SSR and *f*AFLP provided a useful basis for the analysis of an expected population disruption following a resistance breakdown in natural conditions.

Clonal structure of the *virVf* population. The group of *virVf* strains isolated from the *Vf* cv. Judeline demonstrated a clonal structure with a very low genetic diversity and a strong genetic differentiation from the three other *avrVf* subpopulations. Results obtained with the four SSR loci differentiated only two haplotypes among the 36 samples tested from the *Vf* cv. Judeline. In addition, the two haplotypes differed only by two close alleles at a single locus (*Itc1g*), corresponding to an addition of one repeat motif. Because of the high level of mutation of SSR (4,11,13), we argue for a stepwise mutation (41) instead of a recombination event to explain polymorphism observed at this locus. The *f*AFLP technique tested only on DNA originating from monospore isolates confirmed the genetic pattern of this population. This technique, which spans the whole genome, allowed us to differentiate five haplotypes that exhibited 88% genetic similarity. These *virVf*

Fig. 1. Unrooted neighbor-joining tree of four *Venturia inaequalis* populations based on the similarity coefficient calculated with fluorescent amplified fragment length polymorphism data according to Jaccard. Number at the node of the major cluster represents bootstrap value by 100 replications. **P** indicates isolates from the *Vf* cv. Judeline, **PJ** indicates isolates from the non-*Vf* cv. Petit Jaune, **JLN** indicates isolates from the non-*Vf* cv. Juliana, and **Jdr** indicates isolates from the non-*Vf* cv. Judor.

strains remained highly clustered in the dendrogram with a high level of differentiation from the three other populations confirmed by bootstrap analysis. We can therefore conclude that all virVf isolates can be attributed to the same clonal lineage and were derived from one another by minor mutations. This drastic alteration in the diversity of the population fits the concept of the gene-for-gene interaction in which a molecular event occurring in a single individual leads to the selection of one new subpopulation, which is considered as a founder effect. To our knowledge, a founder effect linked to a resistance gene breakdown, in a gene-for-gene perennial pathosystem, has not previously been demonstrated using a population genetic approach.

Unique origin of the loss of avirulence. As far as the origin of the virVf isolates in this orchard population is concerned, the two possibilities are the occurrence of an undetermined mutational event in the indigenous population or gene flow through the introduction of a new strain from another orchard, due to wind-blown spores (23) or to human activities. The virVf isolates seem to exhibit a private allele at locus *Itc1g*. Confirmation of this private allele by increasing the number of samples from this orchard and from other areas in Europe associated with development of new polymorphic microsatellites loci (underway in laboratory) should provide additional evidence regarding the geographical origin of Vf virulence. Evidence of a single cluster of close virulent haplotypes obtained with two independent techniques suggests that the molecular event responsible for virulence against the Vf gene observed in this orchard is unique since the occurrence of multiple independent mutational events would not have led to a single cluster of Vf virulent strains.

The timing of the first event leading to the breakdown of Vf in Europe is difficult but can be evaluated on the scale of this orchard. Because *V. inaequalis* is a heterothallic fungus including two compatibility groups that mate each winter on scabbed leaves on the ground, the strong genetic differentiation between populations clearly indicated that sexual reproduction had not occurred in this orchard with strains present on non-Vf cultivars. The cost of virulence in these isolates is unknown; nevertheless, a change in fitness among virVf isolates cannot be ruled out and should be investigated (2,19). The capacity of virulent strains to compete on non-Vf cultivars may have been reduced, resulting in lower probability of gene exchanges with avrVf strains. If we exclude this last possibility, then we can conclude that strains responsible for scab disease on the Vf cv. Judeline in this orchard have been introduced or have lost their avirulence trait during the very sampling year. Disease observations in the orchard are in accordance with this assumption as no symptoms had been observed on this cultivar before the sampling year.

The founder effect demonstrated in this study is now being assessed in *V. inaequalis* populations in European countries where the Vf resistance gene was also overcome. With barley mildew, Brown et al. (6) has shown that from such a founder effect, evolution forces (recombination and migration) have spread a new virulence widely over Europe. We expect data from our studies on populations of *V. inaequalis* will provide valuable information to develop a strategy for durable apple resistance to scab.

ACKNOWLEDGMENTS

We thank M. Devaux and J. Gaudin for their helpful technical assistance; F. Didelot for collecting strains; and J. P. Paulin, L. Parisi, and M. C. Roland for critical reading of the manuscript.

LITERATURE CITED

- Benaouf, G., and Parisi, L. 2000. Genetics of host-pathogen relationships between *Venturia inaequalis* races 6 and 7 and *Malus* species. *Phytopathology* 90:236-242.
- Bergelson, J., Dwyer, G., and Emerson, J. 2001. Models and data on plant-enemy coevolution. *Annu. Rev. Genet.* 35:469-499.
- Boone, D. M. 1971. Genetics of *Venturia inaequalis*. *Annu. Rev. Phytopathol.* 9:297-318.
- Bowcock, A. M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J. R., and Cavalli-Sforza, L. L. 1994. High resolution of human evolution with polymorphic microsatellites. *Nature* 368:455-457.
- Brown, J. K. M. 1994. Chance and selection in the evolution of barley mildew. *Trends Microbiol.* 2:470-475.
- Brown, J. K. M., Foster, E. M., and O'Hara, R. B. 1997. Adaptation of powdery mildew populations to cereal varieties to durable and non-durable resistance. Pages 119-138 in: *The Gene-for-Gene Relationship in Plant-Parasite Interactions*. I. R. Crute, E. B. Holub, and J. J. Burdon, eds. CAB International, New York.
- Bucheli, E., Gautschi, B., and Shykoff, A. 2001. Differences in population structure of the anther smut fungus *Microbotryum violaceum* on two closely related host species, *Silene latifolia* and *S. dioica*. *Mol. Ecol.* 10:285-294.
- Burdon, J. J. 1997. The evolution of gene-for-gene interactions in natural pathosystems. Pages 245-262 in: *The Gene-for-Gene Relationship in Plant-Parasite Interactions*. I. R. Crute, E. B. Holub, and J. J. Burdon, eds. CAB International, New York.
- Bus, V., Plummer, K., Rikkerink, E., and Luby, J. 2000. Evaluation of the pathogenicity of two scab isolates derived from the Vf resistant apple cultivar Baujade. Pages 231-237 in: *Integrated Control of Pome Fruit Diseases*. L. Parisi, ed. IOBC/WPRS, Gent, Belgium.
- Chen, R. S., and McDonald, B. A. 1996. Sexual reproduction plays a major role in the genetic structure of populations of the fungus *Mycosphaerella graminicola*. *Genetics* 142:1119-1127.
- Edwards, A., Hammond, H. A., Jin, L., Caskey, C. T., and Chakraborty, R. 1992. Genetic variation at five trimeric and tetrameric tandem repeats loci in four human population groups. *Genomics* 12:241-253.
- Flor, H. H. 1971. Current status of the gene for gene concept. *Annu. Rev. Phytopathol.* 9:275-296.
- Forbes, S. H., Hogg, J. T., Buchanan, F. C., Crawford, A. M., and Allendorf, F. W. 1995. Microsatellite evolution in congeneric mammals: Domestic and Bighorn sheep. *Mol. Biol. Evol.* 12:1106-1113.
- Joonsten, M. H. A. J., Cozijnsen, A. J., and De Wit, P. J. G. M. 1994. Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. *Nature* 367:384-387.
- Justesen, A. F., Ridout, C. J., and Hovmoller, M. S. 2002. The recent history of *Puccinia striiformis* f. sp. *tritici* in Denmark as revealed by disease incidence and AFLP markers. *Plant Pathol.* 51:13-23.
- Keller, S. M., Wolfe, M. S., McDermott, J. M., and McDonald, B. A. 1997. High genetic similarity among populations of *Phaeosphaeria nodorum* across wheat cultivars and regions in Switzerland. *Phytopathology* 87:1134-1139.
- Kimura, M., and Crow, J. F. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49:725-738.
- Koller, B., Tenzer, I., and Gessler, C. 2000. SSR analysis of apple scab lesions. Pages 93-98 in: *Integrated Control of Pome Fruit Diseases*. L. Parisi, ed. IOBC/WPRS, Gent, Belgium.
- Leach, J., Vera Cruz, C., Bai, J., and Leung, H. 2001. Pathogen fitness penalty as a predictor of durability of disease resistance genes. *Annu. Rev. Phytopathol.* 39:187-224.
- Le Cam, B., Devaux, M., and Parisi, L. 2001. Specific polymerase chain reaction identification of *Venturia nashicola* using internally transcribed spacer region in the ribosomal DNA. *Phytopathology* 91:900-904.
- Lespinasse, Y. 1989. Breeding pome fruits with stable resistance to diseases, genes, resistance mechanisms, present work and prospects. Pages 100-115 in: *Integrated Control of Pome Fruit Diseases*. C. Gessler, D. J. Butt, and B. Koller, eds. IOBC/WPRS, Gent, Belgium.
- Lespinasse, Y., Durel, C. E., Parisi, L., Laurens, F., Chevalier, M., and Pinet, C. 2000. Durable resistance of apple to scab and powdery-mildew: One step more towards an environmental friendly orchard. Pages 257-260 in: *Integrated Control of Pome Fruit Diseases*. L. Parisi, ed. IOBC/WPRS, Gent, Belgium.
- MacHardy, W. E. 1996. Apple Scab: Biology, Epidemiology and Management. The American Phytopathological Society, St. Paul, MN.
- Majer, D., Mithen, R., Lewis, B. G., Vos, P., and Olivier, R. P. 1996. The use of AFLP fingerprinting for the detection of genetic variation in fungi. *Micol. Res.* 100:1107-1111.
- Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Parisi, L., Durel, C. E., and Laurens, F. 2000. First report on the presence of *Venturia inaequalis* race 7 in french apple orchard. Pages 99-104 in: *Integrated Control of Pome Fruit Diseases*. L. Parisi, ed. IOBC/WPRS, Gent, Belgium.
- Parisi, L., Laurens, F., Didelot, F., Evans, K., Fischer, C., Fouillet, V., Gennari, F., Kemp, H., Lateur, M., Patocchi, A., Schouten, H., and Tsipouridis, C. Geographical distribution of *Venturia inaequalis* strains virulent to the Vf gene in Europe. In: *Integrated Control of Pome Fruit*

- Diseases. Proc. of 6th Workshop, Lindau, Germany, IOBC/WPRS Bulletin. (In Press.)
28. Parisi, L., Lespinasse, Y., Guillaumes, J., and Krüger, J. 1993. A new race of *Venturia inaequalis* virulent to apples with resistance due to the gene Vf. *Phytopathology* 83:533-537.
 29. Perrière, G., and Gouy, M. 1996. WWW-Query: An on-line retrieval system for biological sequence banks. *Biochimie* 78:364-369.
 30. Saitou, N., and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406-425.
 31. Schneider, S., Roessli, D., and Excoffier, L. 2000. Arlequin version 2.00: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
 32. Steele, K. A., Humphreys, E., Wellings, C. R., and Dickinson, M. J. 2001. Support for a stepwise mutation model for pathogen evolution in Australasian *Puccinia striiformis* f. sp. *tritici* by use of molecular markers. *Plant Pathol.* 50:174-180.
 33. Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17:6463-6471.
 34. Tenzer, I., and Gessler, C. 1997. Subdivision and genetic structure of four populations of *Venturia inaequalis* in Switzerland. *Eur. J. Plant Pathol.* 103:565-571.
 35. Tenzer, I., and Gessler, C. 1999. Genetic diversity of *Venturia inaequalis* across Europe. *Eur. J. Plant Pathol.* 105:545-552.
 36. Tenzer, I., Degli Ivanissevich, S., Morgante, M., and Gessler, C. 1999. Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. *Phytopathology* 89:748-753.
 37. Thioulouse, J., Chessel, D., Dolédec, S., and Olivier, J. M. 1997. ADE-4: A multivariate analysis and graphical display software. *Stat. Comput.* 7:75-83.
 38. Thrall, P. H., and Burdon, J. J. 2002. Evolution of gene-for-gene systems in metapopulations: The effect of spatial scale of host and pathogen dispersal. *Plant Pathol.* 51:169-184.
 39. Villaréal, L. M. M. A., Lannou, C., de Vallavieille-Pope, C., and Neema, C. 2002. Genetic variability in *Puccinia striiformis* f. sp. *tritici* populations sampled on a local scale during natural epidemics. *Appl. Environ. Microbiol.* 68:6138-6145.
 40. Vos, P., Hogers, R., Bleecker, M., Reijmans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* 22:4673-4680.
 41. Weber, J. L., and Wong, C. 1993. Mutation of human short tandem repeats. *Hum. Mol. Genet.* 2:1123-1128.
 42. Weir, B. S., and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.
 43. Williams, E. B., and Kuč, J. 1969. Resistance in *Malus* to *Venturia inaequalis*. *Annu. Rev. Phytopathol.* 7:223-246.