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Is virulence phenotype evolution driven exclusively by *Lr* gene deployment in French *Puccinia triticina* populations?

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Abstract

Puccinia triticina is a highly damaging wheat pathogen. The efficacy of leaf rust control by genetic resistance is mitigated by the adaptive capacity of the pathogen, expressed as changes in its virulence combinations (pathotypes). An extensive *P. triticina* population survey has been carried out in France over the last 30 years, describing the evolutionary dynamics of this pathogen in response to cultivar deployment. We analysed the data set for the 2006–2016 period to determine the relationship between the *Lr* genes in the cultivars and virulence in the pathotypes. Rust populations were dominated by a small number of pathotypes, with variations in most of the virulence frequencies related to the corresponding *Lr* gene frequencies in the cultivated landscape. Furthermore, the emergence and spread of a new virulence matched the introduction and use of the corresponding *Lr* gene (*Lr28*), confirming that the deployment of qualitative resistance genes is an essential driver of evolution in *P. triticina* populations. However, principal component analysis (PCA) revealed that certain pathotype–cultivar associations cannot be explained solely by the distribution of *Lr* genes in the landscape. This conclusion is supported by the predominance of a few pathotypes on some cultivars, with the persistence of several other compatible pathotypes at low frequencies. Specific interactions are not, therefore, sufficient to explain the distribution of virulence in rust populations. The hypothesis that quantitative interactions between *P. triticina* populations and bread wheat cultivars—based on differences in aggressiveness—is also a driver of changes in pathotype frequencies deserves further investigation.

KEYWORDS
aggressiveness, *Lr* gene, pathotype distribution, *Puccinia triticina*, varietal landscape, virulence

1 | INTRODUCTION

Leaf rust caused by *Puccinia triticina* is one of the most damaging wheat diseases, with high yield losses worldwide (Savary et al., 2019). This pathogen is a heteroecious biotrophic fungus that can infect bread wheat (*Triticum aestivum*) and durum wheat (*T. durum*) as primary hosts. *P. triticina* requires an alternative host, *Thalictrum speciosissimum*, for its sexual reproduction, and thus for completion of its life cycle (Kolmer, 2013). The alternative host is not naturally present in most places worldwide (including France), and *P. triticina* is therefore generally found exclusively in its uredinial and telial stages on wheat (Kolmer, 2013). The proportion of repeated genotypes is high and there is an excess of heterozygotes in European and French leaf rust populations, confirming the principal role of clonal reproduction (Goyeau et al., 2007; Kolmer et al., 2013).

Genetic resistance is the cheapest and most effective means of limiting leaf rust epidemics. Eighty *Lr* genes, most displaying gene-for-gene (qualitative) interactions, have been identified in wheat cultivars (Kumar et al., 2021). The gene-for-gene relationship depends on the presence of an avirulence (*Avr*) gene in the pathogen (Flor, 1971). A virulence phenotype, also called 'pathotype' or 'race', is defined by a virulence profile: two pathogenic strains are considered to belong to the same pathotype if they have the same combination of virulences. Some *Lr* genes express a partial type of resistance (quantitative or partial) at the adult plant stage. A large proportion of these adult plant resistance (APR) genes, which may have a minor to intermediate effect, appear to be 'race-nonspecific', that is their effect is independent of the virulence phenotype of the pathogen (Lagudah, 2011). However, adult plant resistance genes might express through a strain-specific interaction (Niks et al., 2015). Such resistance reduces disease symptoms, as shown, for example for *Lr34*, which increases the latency period and decreases the production of uredinia (Drijepondt & Pretorius, 1989), or for *Lr46*, *Lr67*, *Lr68*, *Lr74*, *Lr77* and *Lr78* (Huerta-Espino et al., 2020; McIntosh et al., 2020), which increase the latency period and result in the production of fewer, smaller pustules.

Host genetic resistance can be overcome or eroded as a result of the evolution of pathogen populations. Indeed, despite their clonality, *P. triticina* populations are characterized by high diversity, achieved through a combination of asexual reproduction, mutation and migration between wheat-growing areas via the long-distance dispersal of urediniospores. Somatic exchanges in *P. triticina* can also be a significant source of diversity and allelic rearrangements, leading to new combinations of virulence genes (Figueroa et al., 2020). The evolution of *P. triticina* populations is heavily influenced by gene-for-gene interactions, expressed as 'boom-and-bust' cycles of resistance (McDonald & Linde, 2002). Typically, a wheat cultivar with a new single resistance gene is introduced widely into the landscape (boom), and the selection pressure imposed by this resistant cultivar then leads to adaptation of the pathogen population, by mutation, from avirulence to virulence. The new resistance gene loses efficacy as the new virulent population of the pathogen increases (bust) by host resistance gene selection (McDonald & Linde, 2002). In this way, the pathogen has developed virulence towards most of the existing *Lr* genes, but the frequencies and combinations of avirulence genes in pathogen populations vary over space and time (Kolmer, 2013).

The adaptation dynamics of *P. triticina* populations within the varietal landscape result in a rapid, continuous turnover of the predominant virulence phenotypes. Surveys of both host and pathogen populations in the main wheat-growing areas are therefore essential to increase the efficacy and durability of genetic resistances. Leaf rust populations have been monitored for a number of years through field surveys performed around the world (Kosman et al., 2019; Liu & Chen, 2012; Prasad et al., 2017). These surveys aim to evaluate changes in the prevalence and landscape distribution of virulence phenotypes, and to detect any new pathotypes (new virulences and combinations of virulences) that might pose a threat to wheat cultivars with effective leaf rust resistance genes. The data sets generated by these surveys are valuable resources that can help breeders and extension services to propose cultivar deployment strategies both to control leaf rust and to improve the durability of the resistance genes used. Thatcher near-isogenic lines (NILs) were developed to standardize the comparison of virulences between *P. triticina* populations of different geographical origins (McIntosh et al., 1995) and have been used in diverse surveys around the world since 1993.

Most surveys have described only virulence combinations in *P. triticina* populations. Others have linked the virulences in the pathogen population to the *Lr* genes in the cultivars deployed, thereby highlighting the influence of cultivar landscape on the evolution of *P. triticina* populations at large spatiotemporal scales. Such surveys were conducted at the European scale for the 1996–1999 period (Mesterházy et al., 2000), and more recently, from 2018 to 2021, in the European RustWatch research programme (http://rustwatch.au.dk/). Virulence frequencies mostly depend on the leaf rust resistance genes present in the most common varieties in the landscape. Similar results were also obtained in the United States (Kolmer, 2019). In France, the *Lr* gene content of newly released varieties is postulated each year, making it possible to provide a comprehensive and continuous snapshot of the frequency of these genes at landscape level. The *Lr* gene composition of two-thirds of the cultivars present in the French landscape under wheat from 1983 to 2007 has been analysed, with the *Lr13* gene predominating, being found in 67% of cultivars (Goyeau & Lannou, 2011). French leaf rust populations have been monitored for the last 30 years. The populations of strains collected over the 1999–2002 period had highly diverse *P. triticina* virulence phenotypes, with 104 different pathotypes identified (Goyeau et al., 2006). However, the survey revealed domination by a single pathotype (073 100 0) coinciding with a period during which the cultivar landscape was dominated by the cultivar Soissons (up to 40% in 1993). Pathotype 073 100 0 was found to be more aggressive on this cultivar than other virulent pathotypes (Pariaud, Robert, et al., 2009). This adaptation to Soissons, probably due to the absence of an effective *Lr* gene, greater aggressiveness and the high frequency of this cultivar in the landscape, led to the domination of pathotype 073 100 0 from 1999 to 2002. The French wheat landscape tended to diversify thereafter, and the frequency of pathotype 073 100 0 decreased following the decline of Soissons in the landscape (Papaïx et al., 2011). The impact of host quantitative resistance on the evolution of the aggressiveness spectrum of pathogen populations is much less well documented than the impact of *Lr* genes on the prevalence of virulence phenotypes. However, it has been shown that fungal pathogens can evolve and adapt to quantitative resistance through selection for greater aggressiveness (Delmas et al., 2016; Frézal et al., 2018).

The objective of this study was to investigate whether *Lr* gene distribution in the varietal landscape accounts for the prevalence of most of the pathotypes, and/or whether some pathotype–cultivar associations cannot be explained solely by this *Lr* gene distribution. To this end, we determined the impact of the overall composition of the varietal landscape on the evolution of

P. triticina populations over a decade. We gathered data on (a) host, based on postulation of the leaf rust resistance (*Lr*) genes in the cultivars, and (b) pathogen, based on annual surveys over the 2006–2016 period of *P. triticina* virulence phenotypes (pathotypes) across France.

2 | MATERIALS AND METHODS

2.1 | Pathogen population sampling

P. triticina isolates were sampled annually during the 2006–2016 period from a network of nurseries not sprayed with fungicide at about 50 different sites in wheat-growing areas of France. Samples were collected by breeders (the French Wheat Breeders group Recherches Génétiques Céréales, CETAC) and extension services (ARVALIS-Institut du Végétal). The sampling effort focused on the most widely grown bread wheat cultivars, hereafter referred to as major cultivars (i.e., 35 cultivars, each grown on at least 2% of the French wheat-growing area; Table 1), planted in small plots (10–20 m^2) in all nurseries. The cumulative area under wheat planted with these cultivars accounted for between 42.9% and 56.0% of the total bread wheat landscape, depending on the year. For each plot, a few infected leaves of each major cultivar were collected in May or June of each year. A single pustule (uredinium) was selected and urediniospores were collected to obtain one isolate per cultivar and site. Leaf rust samples were also collected from minor bread wheat cultivars (i.e., cultivars grown on less than 2% of the French wheat-growing area) growing in the same nurseries. These minor cultivars were not necessarily present at all sites. The sample obtained from minor cultivars was therefore generally smaller than the sample from major cultivars. Between 75% (2016) and 95% (2010) of the total number of isolates were collected from major cultivars (Table 2).

2.2 | Pathotype determination

The pathotype of each *P. triticina* isolate (i.e., its virulence combination) was determined in planta, by inoculating a differential set of wheat lines as described by Goyeau et al. (2006). Before inoculation, all healthy plant material was grown in cabinets with filtered air, in a glasshouse at temperatures between 15 and 20°C and with a 14-h photoperiod (daylight supplemented with light from 400 W sodium lamps). Infected leaves collected in the field were wiped gently on 7-day-old seedlings of the wheat cv. Michigan Amber treated with 15 ml maleic hydrazide solution (0.25 g/L) to prevent the emergence of secondary leaves and to increase the size of uredinia. Inoculated seedlings were placed for 24 h in a dew chamber at 15°C, then in the glasshouse. One week after inoculation, the seedlings were trimmed so that only one plant with one uredinium remained in each pot. Cellophane bags were then placed over the pots to prevent contamination between isolates. Spores from a single uredinium were then multiplied to produce batches of spores for storage and/or inoculation of a differential set of plant lines. To this end, the spores were collected in a gelatin capsule (size 00) with a cyclone collector 10 days after inoculation. The collected spores were then diluted by adding 0.5 ml of light mineral oil to each capsule, and the resulting spore suspension was sprayed onto 7-day-old Michigan Amber seedlings. The differential sets were sown in pressed-peat pots (3×3 cm^2) containing a commercial compost (peat substrate; Gebr. Brill Substrate), with four seedlings per pot and two pots per line (eight seedlings for each differential line). The differential set consisted of 20 lines (18 Thatcher NILs carrying *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr13*, *Lr14a*, *Lr15*, *Lr16*, *Lr17*, *Lr20*, *Lr23*, *Lr24*, *Lr26* and *Lr37*; CS2A/2 M carrying *Lr28*; and the Australian wheat cv. Harrier carrying *Lr17b*; Singh et al., 2001) and the susceptible wheat cultivar Morocco as a control. After inoculation with each spore suspension, the sets were placed in a dew chamber for 24 h at 15°C and then in a greenhouse compartment maintained at a temperature between 18 and 22°C with a 16-h photoperiod (daylight supplemented with 400 W sodium lamps). The infection type on the differential lines was scored visually 11 days after inoculation, based on the 0–4 scale described by Stakman et al. (1962). Each isolate was assigned a seven-digit code adapted from the scoring system proposed by Gilmour (1973). The 20 lines of the differential set (18 NILs, CS2A/2 M and Harrier) were arranged in six sets of three lines, and one set of two *Lr* genes, with each tested isolate assigned an octal pathotype code.

2.3 | Postulation of resistance genes present in the major wheat cultivars

We postulated the *Lr* genes present in the 35 major French wheat cultivars (Table 1) by performing multipathotype tests in a growth chamber under the conditions described above for pathotype determination. The set of standard *P. triticina* isolates used was changed over time, according to the availability of new virulence combinations, to improve the reliability of postulations. Over the 2006–2016 period, this set consisted of 24 isolates (Table S1). A spore suspension (3 mg of spores per ml of mineral oil) was sprayed onto the first leaves of 12 seedlings of each cultivar, with 0.7 ml suspension applied per tray of 20 cultivars. We included a set of 23 differential cultivars in each test, to check the identity and purity of each isolate and the infection type (Table S2). Infection types were scored with the scale described by Stakman et al. (1962).

2.4 | Data analysis

The diversity of pathotypes in the population was estimated with the Shannon–Weaver index (H'):

$$H' = -\sum \left(p_i \ln\left(p_i\right)\right) \quad (1)$$

where p_i is the frequency of the i^{th} pathotype.

TABLE 1 Bread wheat area (as a percentage) under various cultivars in France during the 2006–2016 period

Cultivar	Registration year	Postulated resistance genes[a]	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Sponsor	1995	*Lr13*	2.0										
Charger	1997	*Lr10*, *Lr13*	5.0										
Autan	2001	*Lr37*	3.0										
Nirvana	2002	*Lr13*, *Lr37*	4.0	2.3									
Orvantis	2000	*Lr10*, *Lr13*, *Lr37*	4.0	3.2									
Isengrain	1997	*Lr14a*	4.0	3.4	3.0								
Soissons	1988	*Lr14a*	4.0	3.8	3.0	2.0							
Sankara	2004	*Lr10*, *Lr13*, *Lr37*	3.0	8.0	9.0	5.0	3.0						
Caphorn	2001	*Lr10*, *Lr13*, *Lr37*	13.0	**13.0**	**12.0**	8.0	5.0	2.5					
Rosario	2004	*Lr10*, *Lr13*, *Lr37*		2.9									
Toisondor	2004	*Lr2a*, *Lr3ka*, *Lr13*		2.6	4.0	3.0							
Mendel	2004	*Lr13*		3.6	4.0								
PR22R58	EU[b]	*Lr1*, (*Lr2c*), (*Lr14a*)			2.0								
Mercato	2002	(*Lr14a*), *Lr37*			3.0	5.0	2.0						
Aubusson	2002	*Lr13*, *Lr14a*, *Lr37*			3.0								
Koreli	2007	*Lr14a*, *Lr37*				2.0	2.0	2.7					
Premio	2007	*Lr14a*, *Lr37*				6.0	8.0	7.6	3.2				
Apache	1998	*Lr13*, *Lr37*	**14.0**	11.0	11.0	**12.0**	**11.0**	**10.1**	**9.3**	**8.0**	6.2	5.0	3.5
Bermude	2007	*Lr13*, *Lr14a*, *Lr37*				3.0	5.0	4.3	4.2	3.0			
Alixan	2005	*Lr13*, *Lr14a*				2.0	3.0	2.9	2.9	2.9	2.8		
Altigo	2007	*Lr3*, *Lr13*, *Lr37*					4.0	6.7	7.5	6.3	4.6		
Arezzo	2008	*Lr10*, *Lr14a*, *Lr37*					2.0	5.6	6.4	7.9	**6.6**	4.7	3.8
Euclide	2007	–						2.9	3.2				
Expert	2008	*Lr1*, *Lr13*						2.7	3.7	3.6	3.1	1,9	
Barok	2009	*Lr10*, *Lr13*, *Lr37*							2.2	2.3	2.4		
Boregar	2008	*Lr13*, *Lr14a*, *Lr37*							2.6	2.8	4.3	4.5	3.8
Solehio	2009	*Lr14a*, *Lr37*								2.5			
Pakito	2011	*Lr13*, *Lr37*								3.6	4.3	3.4	2,3
Cellule	2011	*Lr3*									3.1	6.3	7.9
Rubisko	2012	(*Lr14a*), *Lr28*, (*Lr37*)									6.5	**11.9**	**12.8**
Trapez	2009	*Lr13*, *Lr37*										2.0	
Bergamo	2012	*Lr13*, *Lr14a*, *Lr37*										2.1	2.2

TABLE 1 (Continued)

Cultivar	Registration year	Postulated resistance genes[a]	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Oregrain	2012	*Lr13, Lr28, Lr37*										2.0	3.8
Terroir	2012	*Lr28*											2.0
Fructidor	2014	*Lr13, Lr14a*											5.0
Cumulated area			56.0	53.8	54.0	48.0	45.0	48.0	45.2	42.9	43.9	43.8	47.1

Note: Data from FranceAgrimer. Only bread wheat cultivars accounting for at least 2% of the wheat-growing area for at least 1 year are indicated, with their year of registration and postulated leaf rust resistance genes. The percentage of the area under the most widely grown cultivar for each year is indicated in bold typeface.

[a]Postulations in parentheses would need further validation with appropriate isolates.

[b]EU, European Union.

As H' depends on pathotype frequency, and also on the number of pathotypes (richness), confidence intervals were calculated with the jackknife procedure of the Species-Richness Prediction and Diversity Estimation packages of R software (Zahl, 1977). Evenness was assessed by calculating the E_5 index, also known as the modified Hill's ratio (i.e., the ratio of the number of abundant pathotypes to the number of rarer pathotypes):

$$E_5 = \frac{(G-1)}{(N_1-1)} \quad (2)$$

with $G = 1/\Sigma p_i^2$ and $N_1 = e^{H'}$.

The Stoddart and Taylor index G represents the number of abundant pathotypes and N_1 represents the number of rarer pathotypes. This index is less dependent on pathotype richness than other evenness indices. E_5 approaches 0 as a single pathotype begins to predominate (Alatalo, 1981).

We first estimated the correlation between the frequency of five of the most abundant pathotypes over the entire 2006–2016 period and their frequency on each of eight of the most widely grown French wheat cultivars during the same period, by calculating Pearson's correlation coefficient

$$r = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2 \sum(y-\bar{y})^2}} \quad (3)$$

where x represents the pathotype frequency in the landscape and y its frequency on the eight most widely grown cultivars. Only compatible interactions between pathotypes and cultivars were considered.

We then performed a principal component analysis (PCA) for each year from 2006 to 2016, for (a) major cultivars, defined as a cultivar accounting for more than 2% of the pathogen samples in a given year, that is 19 of the 35 cultivars presented in Table 2; and (b) the most abundant pathotypes, defined as pathotypes with a frequency on the 35 major cultivars exceeding 2% in a given year. The data used for this PCA regrouped only compatible interactions between pathotypes and cultivars. The cultivar Apache was not included in this analysis, because it was considered to display 'neutral' behaviour, based on the results of the previous overall correlation analysis (see the Results and Discussion sections). The PCA of the association between these two components was visualized with the FactoMinerR package (v. 2.4) of R software.

3 | RESULTS

3.1 | Diversity of the *P. triticina* population

Two pathotypes dominated the 2006–2016 period in France: 106 314 0 (virulences 1, 10, 13, 14a, 15, 17, 37) and 166 317 0 (virulences 1, 3, 3bg, 10, 13, 14a, 15, 17, 26, 17b, 37) (Figure 1). From 2006 to 2015, 106 314 0 was the most frequent pathotype, increasing

TABLE 2 Number of isolates collected from the 35 major French wheat cultivars during the 2006–2016 period

Cultivar	Registration year	Postulated resistance genes[a]	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Sponsor	1995	*Lr13*											
Charger	1997	*Lr10*, *Lr13*	26	24	8	1	1						
Autan	2001	*Lr37*		2									
Nirvana	2002	*Lr13*, *Lr37*		1	1								
Orvantis	2000	*Lr10*, *Lr13*, *Lr37*	39	48	16	1	2	1					
Isengrain	1997	*Lr14a*	13	22	10	3	4	4	2				
Soissons	1988	*Lr14a*	37	44	31	23	21	29	8	4	5	4	
Sankara	2004	*Lr10*, *Lr13*, *Lr37*	10	21	18	18	6	6	1				
Caphorn	2001	*Lr10*, *Lr13*, *Lr37*	45	64	41	33	27	18	1				
Rosario	2004	*Lr10*, *Lr13*, *Lr37*	1										
Toisondor	2004	*Lr2a*, *Lr3ka*, *Lr13*			2			2					
Mendel	2004	*Lr13*	4	13	18								
PR22R58	EU[b]	*Lr1*, (*Lr2c*), (*Lr14a*)		8	2	1	1				1		
Mercato	2002	(*Lr14a*), *Lr37*		2									
Aubusson	2002	*Lr13*, *Lr14a*, *Lr37*	11	28	18	23	29	33	21	1			
Koreli	2007	*Lr14a*, *Lr37*	1		1	1		1	1				
Premio	2007	*Lr14a*, *Lr37*			1	29	34	55	45				
Apache	1998	*Lr13*, *Lr37*	47	71	43	44	38	62	50	39	46	45	21
Bermude	2007	*Lr13*, *Lr14a*, *Lr37*					16	38	35	24	31	30	
Alixan	2005	*Lr13*, *Lr14a*	1			1		1	1				
Altigo	2007	*Lr3*, *Lr13*, *Lr37*		1			2	3	35	26	11		
Arezzo	2008	*Lr10*, *Lr14a*, *Lr37*					25	47	49	37	50	60	28
Euclide	2007	–						1					
Expert	2008	*Lr1*, *Lr13*						2	21	16	16	16	5
Barok	2009	*Lr10*, *Lr13*, *Lr37*					1						
Boregar	2008	*Lr13*, *Lr14a*, *Lr37*											
Solehio	2009	*Lr14a*, *Lr37*							37	31	36	41	26
Pakito	2011	*Lr13*, *Lr37*							34	33	38	28	
Cellule	2011	*Lr3*							2	5	45	38	35
Rubisko	2012	(*Lr14a*), *Lr28*, (*Lr37*)							1	7	14	34	18
Trapez	2009	*Lr13*, *Lr37*										1	
Bergamo	2012	*Lr13*, *Lr14a*, *Lr37*											

TABLE 2 (Continued)

Cultivar	Registration year	Postulated resistance genes[a]	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Oregrain	2012	*Lr28*							4	9	17	39	26
Terroir	2012	*Lr28*									13	17	8
Fructidor	2014	*Lr13*, *Lr14a*										1	16
Other minor cultivars			71	69	28	14	10	28	63	63	54	62	62
Total			306	418	238	192	217	331	411	295	377	416	245
Isolates collected on major wheat cultivars (%)			77	83	88	93	95	92	85	79	86	85	75

Note: Only bread wheat cultivars accounting for at least 2% of the wheat-growing area for at least one year are indicated, with their year of registration and postulated leaf rust resistance genes. Cultivars accounting for more than 2% of the pathogen samples in a given year are shown in bold typeface.

[a]Postulations in parentheses would need further validation with appropriate isolates.

[b]EU, European Union.

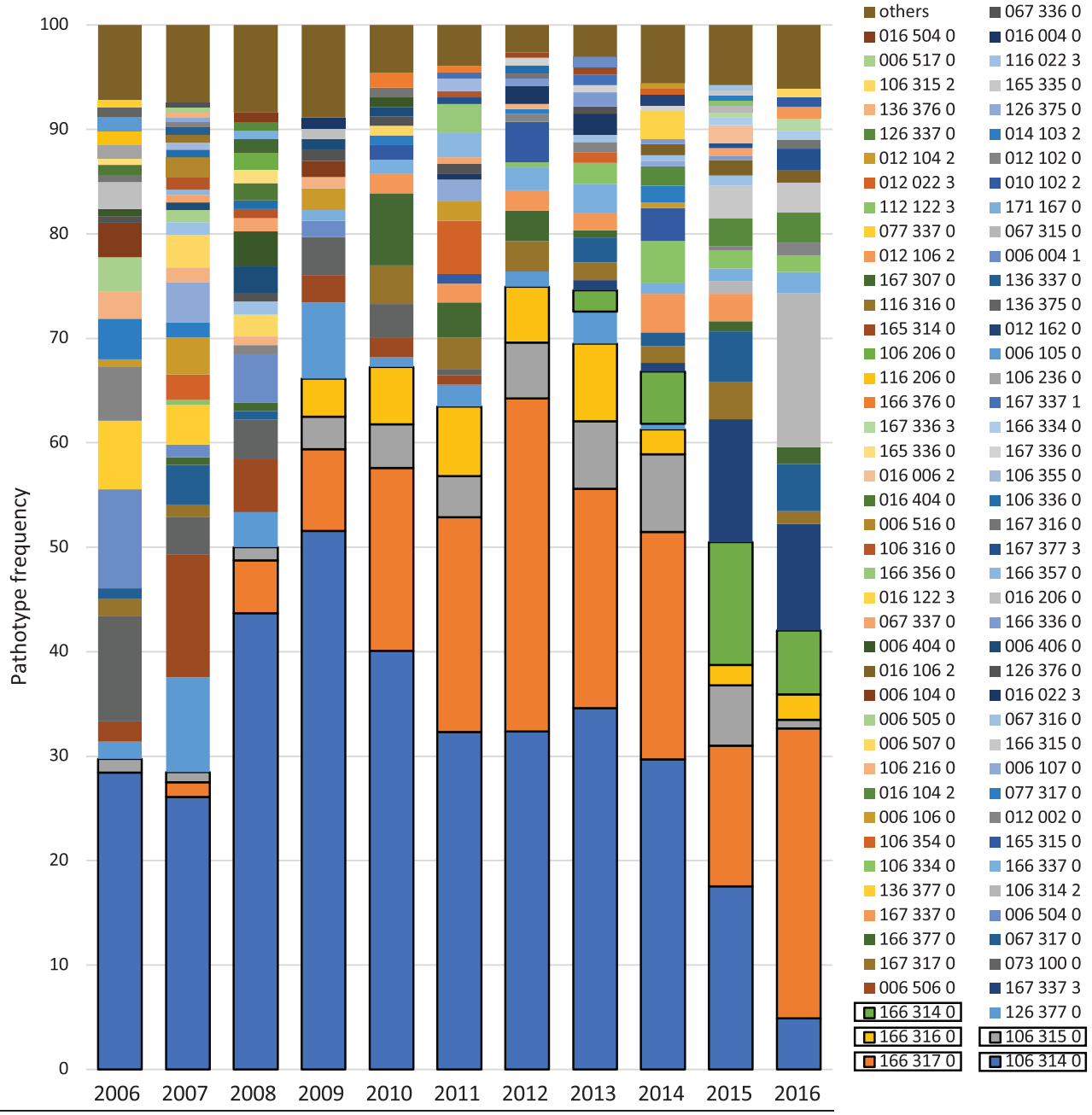
from 28% in 2006 to 51% in 2009, and then decreasing slightly in frequency to reach a plateau at 30%–33% from 2011 to 2014. In 2016, its frequency declined sharply to less than 5%. The other dominant pathotype, 166 317 0, appeared in the landscape in 2007 at a very low frequency (1.4%), then gradually increased to peak at a frequency of 32% in 2012. From 2013 to 2015, the frequency of 166 317 0 decreased to 13%, before increasing to 28% in 2016. Moreover, in 2015, two new pathotypes virulent against *Lr28* appeared (Figure 1): 106 314 2, which had an initial frequency of 1% in 2015, increasing to 14% in 2016; and 167 337 3, which had an initial frequency of 12% in 2015, staying at 10% in 2016. Taken together, these two pathotypes accounted for 24% of the total *P. triticina* population in 2016.

With the exception of the four above-mentioned pathotypes, the frequency of individual pathotypes never exceeded 11% annually. The overall proportion of all other pathotypes decreased from 73% in 2006 and 2007 to 36% in 2012, before increasing again to 50% over the last 2 years of the time period studied (see Table S3 for details). This overall trend was formalized by a decrease in pathotype diversity from 2009 to 2014, with richness (31) and Shannon–Weaver index ($H' = 2.15$) values lowest in 2012 (Figure 1), subsequently increasing until 2015 (richness = 53 and $H' = 3.04$), before declining again in 2016 (richness = 38 and $H' = 2.83$). The evenness of pathotype distribution, represented by the E_5 index, remained stable over the 2006–2016 period (ranging from 0.38 to 0.54), but with a transient decrease in 2008 (0.26) and 2009 (0.29).

3.2 | Changes in virulence frequencies in the pathogen population and prevalence of the corresponding resistance *Lr* genes in the varietal landscape

Virulences could be categorized into three groups according to the pattern of change in their frequencies in the French *P. triticina* population during the 2006–2016 period. The first group (Figure 2a, represented in blue) included virulences against *Lr14a*, *Lr10*, *Lr13*, *Lr37*, *Lr17*, *Lr15* and *Lr1*, with the frequency in the pathogen population increasing rapidly to more than 80% from 2009 to 2016. In terms of the frequency of the corresponding *Lr* genes in the host population (Figure 2b), *Lr13* and *Lr37* were the most represented, with a frequency above 56%, peaking at 72% for *Lr13* in 2006. The frequency of *Lr14a* was initially 16% in 2006 and increased to 46% in 2016. The frequency of *Lr1* was stable at 6% over the 11 years. *Lr10* was the only resistance gene for which a decrease in frequency was observed, from 40% in 2006 to 15% in 2016.

The second group (Figure 2a, represented in green) included virulences against *Lr17b*, *Lr3*, *Lr3bg* and *Lr26*, with a first peak in frequency at the start of the study period (e.g., virulence 17b at 60% in 2007), followed by a transient decrease and then a gradual increase in frequency to between 57% (virulence 26) and 80% (virulence 17b) in 2016. Two of these four genes, *Lr17b* and *Lr3bg*, were never



	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Sample size	306	418	238	192	217	331	411	295	377	416	245
Richness	48	65	46	33	29	37	31	32	46	53	38
Diversity (H')	2.99	3.19	2.75	2.27	2.35	2.57	2.15	2.44	2.67	3.04	2.83
Confidence interval	2.80-3.17	3.01-3.37	2.54-2.95	1.98-2.56	2.15-2.55	2.41-2.73	2.01-2.30	2.27-2.61	2.50-2.84	2.91-3.17	2.62-3.04

FIGURE 1 Frequency of *Puccinia triticina* pathotypes in France during the 2006–2016 period and associated indices of richness (number of virulent phenotypes), diversity (*H'*) and evenness (E_5). The confidence interval for the Shannon–Weaver diversity index (*H'*) was calculated with the jackknife procedure. Pathotypes were characterized on a differential set of 20 *Lr* genes. Five of the most prevalent pathotypes during the period, including 106 314 0 and 166 317 0, are boxed. 'Others' corresponds to pathotypes found only once in the year.

postulated in the cultivars, whereas the frequency of *Lr26* remained stable over the entire period, at less than 3%, and that of *Lr3* increased steadily, from 1% in 2006 to 16% in 2016 (Figure 2b), making *Lr3* the fifth most frequent *Lr* gene in 2016.

The third group (Figure 2a, represented in brown) comprised four virulences against *Lr20*, *Lr3ka*, *Lr2c* and *Lr23*, with frequencies that remained below 30%. Of the four corresponding *Lr* genes, only *Lr3ka* and *Lr2c* were postulated in the cultivars,

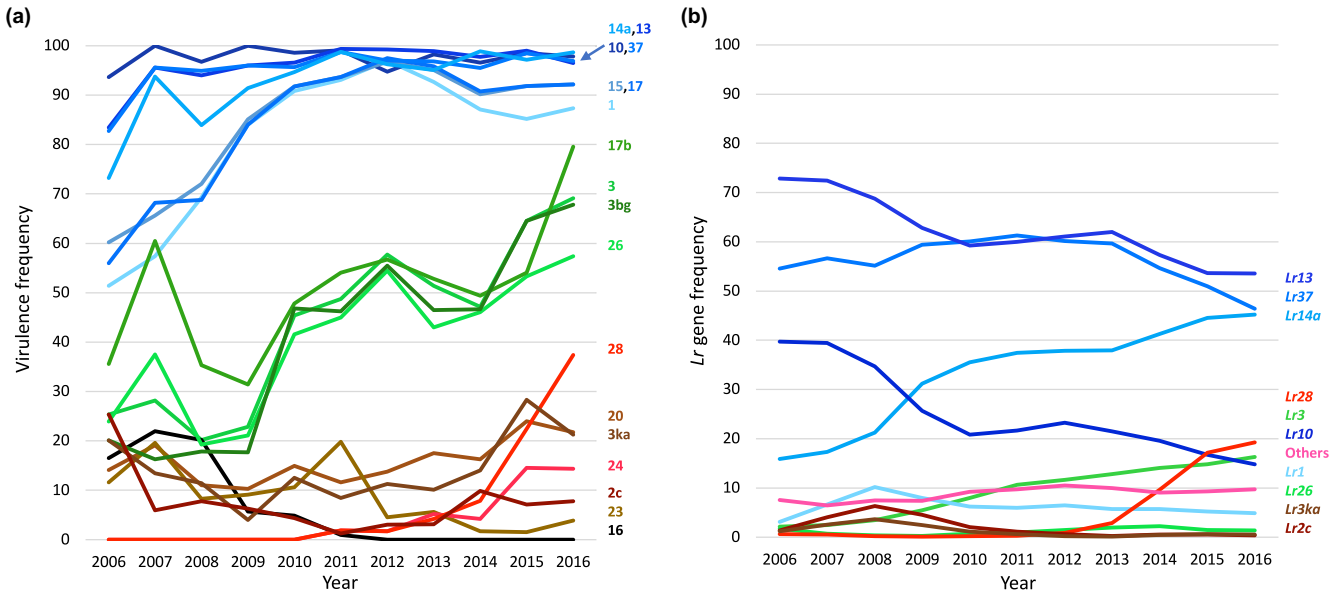


FIGURE 2 Changes in virulence frequencies (a) in the *Puccinia triticina* population and (b) in the frequencies of the corresponding *Lr* resistance genes in the host population in the French landscape during the 2006–2016 period. Pathotypes were identified on a differential set of 20 *Lr* genes, and *Lr* gene combinations in the cultivars were postulated based on a set of standard isolates. 'Others' corresponds to cultivar mixtures, unidentified cultivars, and cultivars carrying unidentified genes.

TABLE 3 Pearson's correlation coefficient between the frequency of the five most prevalent pathotypes in the landscape during the period 2006–2016, and their frequency on eight of the major wheat cultivars

Pathotype	Virulences (against the corresponding *Lr* genes)	Cultivar							
		Arezzo	Apache	Expert	Aubusson	Premio	Bermude	Altigo	Cellule
106 314 0	1, 10, 13, 14a, 15, 17, 37	0.88*	0.86*	0.76	0.16	1.00*	0.57	–	–
106 315 0	1, 10, 13, 14a, 15, 17, 26, 37	0.57	0.90*	−0.53	0.44	−0.48	0.93*	–	–
166 314 0	1, 3, 3bg, 10, 13, 14a, 15, 17, 37	0.87	0.92*	0.87	–	–	–	0.78	1.00
166 316 0	1, 3, 3bg, 10, 13, 14a, 15, 17, 17b, 37	0.12	0.67	0.70	0.73	0.46	−0.23	0.96	1.00
166 317 0	1, 3, 3bg, 10, 13, 14a, 15, 17, 26, 17b, 37	0.61	0.82*	0.89	0.85	0.98	0.66	0.69	0.85

Note: *Lr* gene combination: Arezzo (*Lr10*, *Lr14a*, *Lr37*), Apache (*Lr13*, *Lr37*), Expert (*Lr1*, *Lr13*), Aubusson (*Lr13*, *Lr14a*, *Lr37*), Premio (*Lr14a*, *Lr37*), Bermude (*Lr13*, *Lr14a*, *Lr37*), Altigo (*Lr3*, *Lr13*, *Lr37*), Cellule (*Lr3*). –, Pathotype never found on the cultivar. * Significant coefficient, $p < 0.01$.

at a very low frequency: less than 7% for *Lr2c* and 3% for *Lr3ka* (Figure 2b).

Virulence against *Lr16*, which had a frequency of about 16% at the start of the study period (Figure 2a), was no longer detected after 2011, and *Lr16* was never postulated in the cultivars.

New virulences emerged in 2011 (Figure 2a, represented in red), increasing in frequency until 2016, to values of 15% for virulence 24 and 37% for virulence 28. The frequency of *Lr24* in the cultivars remained very low, at less than 1% (data not shown). The frequency of *Lr28* in the cultivars increased steadily, from 1% in 2012 to 19% at the end of the study period (Figure 2b, represented in red).

Part of the landscape, consistently about 10% over the 2006–2016 period, was planted with cultivar mixtures, unidentified cultivars, or cultivars carrying unidentified genes (Figure 2b). Over the 2006–2016 period, more than 81% of the pathotypes, including the major pathotypes 106 314 0 and 166 317 0, carried virulences 10, 13, 14a and 37 (Table S4). Thus, these pathotypes were virulent on the most frequent *Lr* genes, and consequently on 28 of the 35 most widely grown cultivars (Table 1). In addition, pathotype 166 317 0 carried virulence 3, leading to a compatible interaction with 30 of the 35 most widely grown cultivars.

3.3 | Pattern of association between *P. triticina* pathotypes and wheat cultivars

The frequency of four of the five most frequent pathotypes in the landscape (106 314 0, 106 315 0, 166 314 0 and 166 317 0) was correlated with their frequency on Apache over the entire 2006–2016 period ($p < 0.01$; Table 3), meaning that their frequencies evolved in

the same way and proportion in the landscape and on this cultivar. Apache was the only cultivar for which this was the case over the whole period. This result justified a PCA excluding Apache, which was considered to display neutral behaviour (see Discussion section), to make it possible to detect associations.

The frequency in the landscape of pathotype 106 314 0, which is avirulent on cultivars Altigo and Cellule carrying *Lr3*, was significantly correlated with its frequency on cultivars Arezzo, Apache and Premio, but not with its frequency on Expert, Aubusson or Bermude, over the entire 2006–2016 period (Table 3). The PCA refined the association between the major cultivars and the most abundant pathotypes for each year of the 2006–2016 period separately, and thus independently of changes in the pathogen population that could potentially alter or hide such a relationship in an overall analysis of the whole data set. The first dimension of the PCA explained at least 48.9% and, at most, 79.1% of the variability, depending on the year considered (Figure 3). Based on the PCA results, we distinguished a group of cultivars associated with pathotype 106 314 0 (virulences 1, 10, 13, 14a, 15, 17, 37) from a group of cultivars associated with 166 317 0 (virulences 1, 3, 3bg, 10, 13, 14a, 15, 17, 37). Pathotype 106 314 0 was always associated with several cultivars, the precise identity of which depended on the year: Aubusson (*Lr10*, *Lr13*, *Lr37*), Sankara (*Lr10*, *Lr13*, *Lr37*) and Charger (*Lr10*, *Lr13*), from 2006 to 2009, then Arezzo (*Lr10*, *Lr14a*, *Lr37*) in 2010 and 2011, and subsequently Bermude (*Lr10*, *Lr13*, *Lr14a*, *Lr37*) and Aubusson until 2015. In 2015, new associations with cultivars Pakito (*Lr13*, *Lr37*), Solehio (*Lr14a*, *Lr37*) and Expert (*Lr1*, *Lr13*) were highlighted. In 2016, 106 314 0 (virulences 1, 10, 13, 14a, 15, 17, 37) was weakly associated with only one cultivar, Expert.

The frequency of pathotype 166 317 0 (virulences 1, 3, 3bg, 10, 13, 14a, 15, 17, 26, 17b, 37) in the landscape was correlated with its frequency on only one cultivar, Apache, over the entire 2006–2016 period (Table 3). PCA showed that pathotype 166 317 0 was mostly associated with a single cultivar in each year (Figure 3), but that this cultivar differed between years during the 2006–2016 period: Caphorn (*Lr10*, *Lr13*, *Lr37*) from 2008 to 2011, then Altigo (*Lr3*, *Lr13*, *Lr37*) in 2012–2013, and finally, Cellule (*Lr3*) in 2014–2015. In 2016, unlike other years, this pathotype was associated with several cultivars, Cellule, Fructidor (*Lr13*, *Lr14a*), Arezzo (*Lr10*, *Lr14a*, *Lr37*) and Solehio (*Lr14a*, *Lr37*).

Pathotypes 167 337 3 and 106 314 2 emerged in 2015 and appeared to be strongly associated with new cultivars carrying the *Lr28*

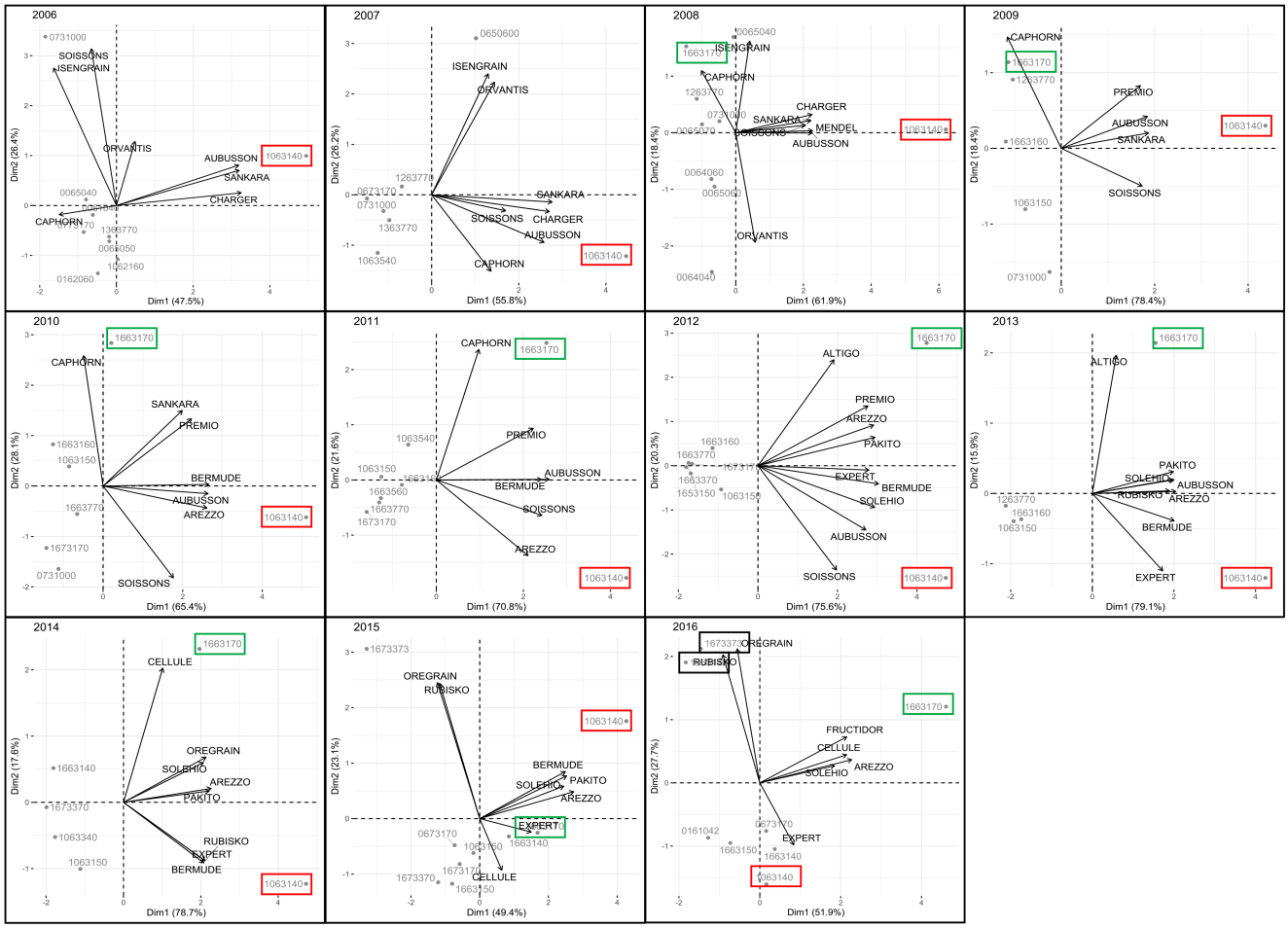


FIGURE 3 Principal component analysis (PCA) representing 19 of the 35 most widely grown cultivars (Table 1) as variables and *Puccinia triticina* pathotypes as individuals for each year between 2006 and 2016. The two most prevalent pathotypes, 106 314.0 and 166 317 0, are boxed in red and green, respectively. The two pathotypes that emerged in 2015, 167 337 3 and 106 314 2, are boxed in black.

resistance gene—Oregrain (*Lr13*, *Lr28*, *Lr37*) and Rubisko (*Lr28*)—in 2015 and 2016 (Figure 3).

Others pathotypes presented in the PCA almost never showed any association with any cultivars despite compatible interactions with the majority of tested cultivars, except for interactions involving *Lr28*.

4 | DISCUSSION

In this study, we analysed the changes in pathotypes in the French *P. triticina* population over an 11-year period. We found that changes in pathotype distribution depended on the *Lr* genes present in the cultivar landscape, consistent with previous findings. However, we also found that *Lr* genes were not sufficient to fully account for the observed pathotype distribution. This conclusion, which may appear counterintuitive, is robust, given the extensive nature of our survey; samples of *P. triticina* were collected from the most common wheat cultivars in a network of about 50 nurseries distributed throughout the main wheat-growing areas in France. The diversity and pathotype frequency found in such nurseries are known to reflect the situation in the commercial wheat-field landscape (Kolmer, 1992).

Changes in the frequency of pathotypes in the *P. triticina* population appeared to depend on the changes in and prevalence of *Lr* genes in the varietal landscape. An analysis of the distribution of pathotypes frequencies revealed that one pathotype, 106 314 0, virulent against *Lr1*, *Lr10*, *Lr13*, *Lr14a*, *Lr15*, *Lr17* and *Lr37*, predominated in France from 2006 to 2016. Three of these virulences matched the most prevalent *Lr* genes in the French landscape: *Lr13*, *Lr14a* and *Lr37*. This finding suggests that this virulent pathotype was subject to active selection due to the presence of these three *Lr* genes in the cultivars, and thus the frequency of these cultivars in the landscape. It has already been shown that *Lr* genes drive the selection of pathotypes. For instance, *Lr39* was widely used in the United States, leading to the selection of *P. triticina* populations in which more than 50% of the strains were virulent against this gene (Kolmer, 2019). Older data sets available in France suggest that the *Lr* genes present in the varietal landscape drive the selection of pathotypes; from 1999 to 2002, *Lr13* and *Lr14a* were the two most frequent resistance genes present in the cultivars grown, and corresponding virulences were the two most common in the *P. triticina* population (Goyeau et al., 2006).

The group of pathotypes carrying virulence against *Lr3*, including about 30 different pathotypes, steadily increased in frequency over the 2006–2016 period. This may reflect the strong pressure imposed by the increasing frequency of the *Lr3* resistance gene in the varietal landscape. As this virulence was carried by pathotype 166 317 0 (virulences 1, 3, 3bg, 10, 13, 14a, 15, 17, 26, 17b, 37), but not by 106 314 0 (virulences 1, 10, 13, 14a, 15, 17, 37), the presence of *Lr3* in the landscape probably conferred an advantage on 166 317 0. This finding is consistent with the outputs of the PCA for 2012 data, which highlighted an association of pathotype 166 317 0 with cultivars Altigo and Cellule carrying *Lr3*. *Lr3* was deployed for the first time in France in two cultivars registered in 1988, Génial and Louvre, and subsequently in six other cultivars registered in 1998 (Goyeau & Lannou, 2011). This deployment of cultivars carrying *Lr3* resistance was followed by a gradual increase in the frequency of virulent pathotypes, including 166 317 0.

New virulences against *Lr24* and *Lr28* appeared in 2011. *Lr24* had been present in the varietal landscape since 2006, but at a very low frequency, remaining below 1% until 2012. The increase in the frequency of virulence against *Lr28* immediately followed the registration of cultivars carrying *Lr28* but began before the widespread deployment of these cultivars (<1% in 2011 and 2012; >9% in 2014), suggesting that this resistance gene acted as a strong selective driver of the evolution of the *P. triticina* population. *Lr28* resistance was overcome within 2 years (2014–2015), with a large decrease of its efficacy in France. A similar pattern was observed in the Czech Republic, where *Lr28* conferred high levels of resistance (rated 7.0) to cultivars widely deployed in 2013, but displayed a rapid loss of efficacy, with resistance levels falling to 2.7 for these cultivars in 2015 (Hanzalová et al., 2021). *Lr* genes are generally rapidly overcome in wheat-growing areas worldwide. For example, *Lr3ka*, *Lr11* and *Lr24* were overcome within 2 years of their introduction in North American cultivars (Kolmer, 1996).

Some virulences, such as those directed against *Lr15* and *Lr16*, were detected in the *P. triticina* populations sampled despite the absence of the corresponding *Lr* genes from the cultivars used during the 2006–2016 period. These examples illustrate the general conclusion recently drawn by Kolmer (2019) that resistance genes select strains with specific virulence, but that this virulence may already exist in the pathogen population. The indirect selection, or at least an absence of counterselection, of these virulence phenotypes considered unnecessary for the pathogen, may come under a hitchhiking effect, due to the clonality of *P. triticina* (de Vallavieille-Pope et al., 2011).

This survey clearly showed that the frequency of pathotypes is strongly influenced by *Lr* gene prevalence in the French varietal landscape. However, our extensive data set analysis could not fully account for the observed patterns within the pathogen population, suggesting that there is selection pressure on traits other than virulence.

The frequencies of four of the five most prevalent pathotypes were correlated with the frequency of Apache, which was the only cultivar involved in a significant correlation with more than one pathotype. The five other tested cultivars (Arezzo, Expert, Aubusson, Premio, and Bermude) were, like Apache, susceptible to nearly every pathotype, but involved in a correlation with at most one pathotype. The overall prevalence of these four pathotypes in the French varietal landscape was thus quite similar to their frequency on Apache, meaning that their frequencies evolved in the same way in the landscape and on this cultivar. This is consistent with the previous finding that no pathotype was preferentially associated with Apache in French wheat-growing areas generally (Papaïx et al., 2011). Unlike the other cultivars considered, Apache did not

exert the significant selective pressure that could be expected from the *Lr* genes it carries. This cultivar was thus considered neutral and was not included in the PCA.

Two pathotypes predominated in France during the 2006–2016 period. First, 106 314 0 alone, then 106 314 0 in codominance with 166 317 0, and finally, 166 317 0 alone. Overall population diversity decreased when these two pathotypes were codominant in the varietal landscape (together accounting for more than half the sampled isolates). These dynamics for the 2006–2016 period differ from those in France between 1999 and 2002 described in a previous study (Goyeau et al., 2006), suggesting that *P. triticina* diversity is greater when there are two codominant pathotypes rather than a single dominant pathotype.

PCA revealed that pathotype 106 314 0 was associated, almost always alone, with different cultivars (11 in total) each year from 2006 to 2014, despite the presence of other compatible pathotypes. This pattern reflects generalist behaviour, that is, an adaptation of this pathotype to several host genetic backgrounds. Other pathotypes carrying virulence genes theoretically enabling them to attack the same cultivars were identified through the annual survey but were never present at a meaningful frequency (Table S3). For instance, while pathotype 166 317 0 predominated at the end of the 2006–2016 period, other pathotypes virulent against *Lr3* were found at a much lower frequency on cultivars carrying *Lr3*. The higher prevalence of 106 314 0 and 166 317 0 cannot, therefore, be explained exclusively by their combination of virulences. Biases due to the focusing of sampling on specific cultivars (the most widely grown) may partly account for such effects but are unlikely to provide a full explanation. Thus, the selective pressure exerted by *Lr* genes is not the only evolutionary force driving the dynamics of the adaptation of *P. triticina* populations to their host populations at large spatial scales.

A previous study, over the 1999–2008 period in France, demonstrated that some *P. triticina* pathotypes were preferentially associated with certain cultivars while being compatible with most of the cultivars deployed in the landscape (Papaïx et al., 2011). In this previous study, the resistance level of a cultivar was linked to the frequency of the most aggressive pathotype of all the compatible pathotypes present in the *P. triticina* population. During the period considered by this previous study, the rust population was dominated by one pathotype, 073 100 0, associated with the wheat cultivar Soissons, which was the most widely grown cultivar in France until 1999 (reaching a maximum of 40% of the total area under wheat in 1993). Pathotype 073 100 0 was more aggressive on Soissons than other common virulent pathotypes able to infect this cultivar. This difference in aggressiveness, characterized by a larger uredinium size and higher level of spore production per square millimetre of sporulating tissue, explained this preferential cultivar-pathotype association (Pariaud, Robert, et al., 2009). Accordingly, as Soissons was the most widely grown cultivar, the high frequency of the pathotype 073 100 0 on this cultivar was considered likely to account for its overall prevalence at the landscape scale. A study involving pathotype 106 314 0, similar to the study conducted on pathotype 073 100 0, would be needed to establish if pathotype 106 314 0 is more aggressive on susceptible cultivars, as compared to other compatible pathotypes.

Moreover, Papaïx et al. (2011) highlighted an association of pathotype 106 314 0 with the wheat cultivar Caphorn, even though this pathotype is considered avirulent on this cultivar at the adult plant stage (unpublished data). This illustrates that during a severe epidemic, uredinia of an incompatible pathotype can, indeed, be found on a cultivar (Table S3). We did not consider interactions of this type, between incompatible pathotypes and cultivars, in this study.

At the start of this century, the 10 most widely grown cultivars in France accounted for about 70% of the total area under wheat. This percentage dropped between 43% and 56% during the 2006–2016 period (Table 1). The varietal landscape has thus tended to become more diversified, potentially accounting for the predominance of a more generalist pathotype. The generalist behaviour of such a pathotype could be related to its aggressiveness, the quantitative component of pathogenicity (Lannou, 2012; Pariaud, Ravigné, et al., 2009). Aggressiveness is considered less dependent on host genetic background. Thus, a *P. triticina* pathotype with a higher aggressiveness on a range of cultivars would be fitter in a diversified or heterogeneous biotic environment (Kröner et al., 2017). The association between pathotype 106 314 0 and the most widely grown cultivars was not related to the compatibility of its virulence profile with the combination of *Lr* genes in these cultivars. It therefore seems likely that aggressiveness traits (i.e., latency period, sporulating capacity and infection efficiency) played a significant role. The selection of groups of strains with higher aggressiveness, as a mechanism driving the evolution of pathogen populations, has been demonstrated in other pathosystems, through empirical evidence of local adaptation (Delmas et al., 2016; Kröner et al., 2017). For instance, in the *Phytophthora infestans* population, a detached-leaflet assay showed that the most aggressive genotypes on a potato cultivar (genotype with the shortest latency period or highest infection efficiency) tended to be those selected by the same cultivar in the field. Moreover, differences in aggressiveness between isolates were amplified on cultivars with the highest levels of partial resistance (Young et al., 2018). In *Plasmopora viticola*, strains collected from resistant grapevine cultivars were more aggressive than isolates collected from susceptible hosts, demonstrating the occurrence of selection for greater aggressiveness (Delmas et al., 2016).

The hypothesis of dual selection, based on both qualitative and quantitative interactions between *P. triticina* and bread wheat populations, is consistent with the dynamics described here. Unlike pathotype 106 314 0, pathotype 166 317 0 was associated with only one cultivar each year (except in 2016), first with Caphorn, then with Altigo and finally with Cellule. The pathotype 166 317 0 is virulent against *Lr3*, *Lr10*, *Lr13* and *Lr37*, and would therefore theoretically be able to overcome the *Lr* genes carried by Caphorn and Altigo. Even if these cultivars carry *Lr* resistance genes not yet identified, the pathotype 166 317 0 is also virulent to these putative genes, as its interaction observed with Caphorn

and Altigo is compatible. However, these two cultivars have high levels of resistance to leaf rust (and, indeed, the leaves sampled from these two cultivars carried only a few uredinia). We therefore hypothesize that the isolates found on these two cultivars had undergone selection for greater aggressiveness (relative to other strains, when expressed on susceptible cultivars) and that the selective dynamics had turned to their advantage not only at the field scale, but also at the landscape scale. Further experimental studies based on tests of local adaptation (isolate × cultivar cross-inoculations) by comparing aggressiveness traits would be useful to validate this hypothesis and to quantify its potential effects on population evolutionary dynamics. The neutral status of Apache renders this cultivar particularly interesting for comparisons of pathotype aggressiveness without a selection effect of the tested cultivar.

This study demonstrated that *Lr* genes impact *P. triticina* populations. Indeed, as was the case for *Lr28*, new pathotypes adapted to *Lr* genes appeared in fungal populations shortly after the introgression of these genes into cultivars. However, the *Lr* genes in the most frequently grown cultivars could not explain the prevalence of only two pathotypes in the landscape, despite the presence of other compatible pathotypes.

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CONFLICT OF INTEREST

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

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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