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Clonality and host selection in the wheat pathogenic fungus *Puccinia triticina*

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Abstract

Clonal reproduction in *Puccinia triticina*, the cause of wheat leaf rust, has long been hypothesized but has never been demonstrated. Using a population genetics approach and microsatellite markers, we analysed genetic diversity of this fungus at each level of genome organisation. Sampling included isolates from two field populations growing on two cultivars carrying specific resistance genes, completed with isolates representing the main pathotypes identified from a national survey. For the two cultivars, populations differentiated according to the distribution of their genotypes and pathotypes. There was a high proportion of repeated genotypes, combined with a significant linkage disequilibrium and a strong negative value for F_{IS}. These three factors, especially heterozygote excess, strongly support the hypothesis of a high rate of clonal reproduction. Each pathotype matched a unique multilocus genotype, except for a few isolates, which were taken to be mutants of the dominant genotype. We discussed the strong relationship between pathotypes and genotypes as the consequence of clonal reproduction combined with a strong selection exerted by host cultivars.

Keywords: Heterozygosity; Population; Recombination; Mutation; Pathotype; Microsatellite; Wheat leaf rust; Host cultivar

1. Introduction

In several plant pathogenic fungi, reproduction is thought to be mainly clonal (Milgroom, 1996). Clonality combined with a strong selection exerted by host cultivars may increase the frequency of the fittest genotypes (McDonald and Linde, 2002). This combination of clonality and selection should leave little room for the emergence of new genotypes, except through mutation. However, rare events of recombination, such as cryptic sexual reproduction or gene conversion can have a drastic effect on the reshuffling of genetic material (Burt et al., 1996; Bengtsson, 2003), resulting in a faster spread of virulence genes and an easier emergence of new virulence combinations than through mutation alone (Awadalla, 2003). Thus, accurate knowledge of the rate of clonal reproduction in populations of plant pathogenic fungi is crucial if pathogen control is to be made all the more efficient (Milgroom and Fry, 1997; McDonald and Linde, 2002).

Direct observation of sexual stage (or a lack thereof) cannot provide a reliable estimate of the clonal rate in natural populations (Halkett et al., 2005). Inferences on clonal structure of pathogen populations are better assessed using molecular markers (Taylor et al., 1999), e.g. through frequencies of multilocus genotypes (Milgroom, 1996). Recent advances in population genetics of clonal organisms have revealed that heterozygote excess increases with clonal rate (Balloux et al., 2003). This clear signal of a lack of sexual reproduction has been rarely studied in the case of diploid or dikaryotic plant pathogenic fungi; moreover, it has not yet been discussed in light of recent theory about heterozygosity in clonal populations. Given adequate sampling strategies, suitable molecular markers and a clear statistical

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guideline (Halkett et al., 2005) it is possible to estimate the heterozygote excess, therefore the rate of clonal reproduction (e.g., Nebavi et al., 2006).

Cereal rust fungi display complex and various life cycles. These dikaryotic basidiomycetes are heteroaecious and complete their sexual cycle on an alternate or aecidial host, e.g., *Puccinia coronata* in most North American areas (Simons, 1985). However, no alternate host has ever been found for *Puccinia striiformis*, which is therefore considered to undergo asexual reproduction only (Stubbs, 1985). *Puccinia triticina* is in between. Its sexual stage has been extensively sought out in the field, but found only occasionally on *Thalictrum flavum* ssp. *glaucum* in Portugal (D'Oliveira, 1940; D'Oliveira and Samborski, 1964), in Italy (Casulli and Siniscalco, 1987) in Spain (Salazar cited in Young and D'Oliveira, 1982), and in The United States (Levine and Hildreth, 1957). One example of somatic recombination for *P. triticina* in the field was reported (Park et al., 1999), but the rate remains unknown.

Puccinia triticina causes wheat leaf rust and is present nearly everywhere that wheat (*Triticum aestivum*) is grown (Samborski, 1985). More than 50 specific resistance genes to *P. triticina* have been characterized, reviewed in Kolmer (1996), and updated on http://www.cdl.umn.edu, but most of them were overcome within a few years (Kolmer, 2001) when used alone or even in combination. The proposed mechanisms for variation in *P. triticina*, inferred from the distribution of virulence phenotypes, and more recently from RAPD and AFLP genotypes, are mutation in clonally reproducing populations, accompanied by selection exerted by host cultivars (Kolmer, 2001). However, the samples analysed (i.e., collections of isolates from various years and regions) and the dominant markers used did not allow a formal validation of the hypothesis of clonal reproduction, and therefore could not produce an estimation of the rate of clonal reproduction in *P. triticina* populations.

French populations of *P. triticina* have been characterized according to their pathotype distribution (Goyeau et al., 2006), a pathotype being defined by its avirulence/virulence formula on a set of differential hosts carrying wheat leaf rust resistance genes (*Lr* genes). As host selection has been shown to strongly shape French population structure, based on pathotype frequencies, in this study a rust population is defined as all the isolates collected from a given wheat field planted with a single cultivar (i.e., host genotype).

Based on the pathotype structure described in a previous paper (Goyeau et al., 2006), we focused our study on two host cultivars and a sub-sample of isolates from a national survey. Using recently developed microsatellite markers (Duan et al., 2003), we assessed the population structure of *P. triticina* in two French wheat fields, and in particular (1) we estimated the extent of clonal reproduction within these populations and (2) we investigated the interaction between clonality and selection exerted by the host by examining the link between genotypic and phenotypic (pathotypes) measurements.

2. Material and methods

2.1. Sample collection

Isolates of *P. triticina* were collected at the end of May 2001 from untreated 0.5-Ha plots of Soissons (*Lr14a*) and Trémie (*Lr10*, *Lr13*, Adult Plant Resistance) cultivars, in the south-western French towns of Flamarens and Ondes, respectively, and located 70 km from each other. Soissons and Trémie were the two most widely grown cultivars in France in three out of the four years preceding 2001. For Soissons, a single infected leaf was collected from each of 198 plants located 3 m apart from each other. A single-uredinium isolate was then produced from each of the 198 leaves. For Trémie, a single infected leaf was collected from each of 100 plants located according to a 6×2 m grid. The spores from the 100 leaves were then bulked, and 50 single-uredinium isolates were produced by inoculating the bulked spores at a low density on susceptible wheat seedlings. The pathotype of all isolates was determined. A sub-sample of isolates was selected for microsatellite genotyping, which included all isolates belonging to low-frequency pathotypes and a random sample of isolates belonging to the most frequent pathotypes (Table 1). In total, 195 and 49 isolates were assigned a pathotype, and 66 and 45 were genotyped for Soissons and Trémie respectively. This sample was completed by 40 "reference" isolates collected during a leaf rust national survey conducted in France between 1997 and 2003. These isolates, from various geographic origins and collected on different dates, were chosen to represent the main pathotypes identified on the most common cultivars. All 40 of these isolates were tested again for their pathotypes and were genotyped. Overall, 283 isolates were phenotyped (identification of the pathotype) and 151 were genotyped. Procedures for single-uredinium isolation and spore increase are described in Goyeau et al. (2006).

2.2. Phenotypic analysis

Pathotype identification was replicated at least twice in independent experiments by inoculating a differential set of wheat cultivars comprising 17 Thatcher differential lines with genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr3bg, Lr3ka, Lr10, Lr13, Lr14a, Lr15, Lr16, Lr17, Lr20, Lr23, Lr26, Lr37*, the Australian cultivar Harrier carrying *Lr17b*, and the susceptible control Morocco. After inoculation of the first seedling leaf with a spore suspension, the sets were placed for 24 h in a dew chamber at 15 °C, and then for 9 days in a greenhouse maintained at 18–22 °C with a 14 h photoperiod of daylight supplemented with 400 W sodium vapor lamps. Infection types on the differentials were read 10 days after inoculation according to Stakman et al. (1962). The 18 differential isolines were arranged in six sets of three, in order to assign an octal pathotype code to each isolate, according to Goyeau et al. (2006).