Title

Stability in the genetic structure of a *Zymoseptoria tritici* population from epidemic to interepidemic stages at a small spatial scale

Running head

Z. tritici population stability

Authors

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Abstract

Subpopulations of the wheat pathogen *Zymoseptoria tritici* (26 sample groups composed of 794 pure strains) were collected in two nearby wheat fields, during both epidemic and interepidemic periods of three successive years (2009-2013). The alternative presence of wheat debris allowed taking into account different forms (ascospores *vs.* pycnidiospores) of inoculum and, thus, its putative origin (local *vs.* distant). We used a molecular epidemiology approach, based on population genetic indices derived from SSR marker analysis. The aim was to describe putative changes in the neutral genetic structure and diversity of these subpopulations over the time-course of several epidemics, in two close fields, *i.e.* at a spatiotemporal scale consistent with epidemiological observations, and to determine the possible structuring factors. Genetic structure was stable over time (within and between years) and between fields. All subpopulations displayed very high levels of gene and genotypic diversity. The low levels of linkage disequilibrium and the low clonal fraction, and the similar frequencies of the two mating types in most subpopulations were consistent with the regular occurrence of sexual reproduction in the two fields over the epidemic and interepidemic stages. In conclusion, at this fine spatiotemporal scale, we found that the period, the nature of inoculum and its putative origin had little effect on the short term evolution of the local population, with population size and diversity apparently large enough to prevent genetic drift, and with a major contribution of migration between and within plots to the stabilization of genetic diversity.

Keywords

Diversity, molecular epidemiology, population structure, *Mycosphaerella graminicola*, primary inoculum, Septoria tritici blotch.

Introduction

Septoria tritici blotch can cause yield losses of 10-40% (Eyal *et al*., 1987; HGCA, 2012) on wheat (*Triticum aestivum*) crops in several wheat-growing areas worldwide. This disease is caused by the hemibiotrophic, heterothallic, ascomycete fungus *Zymoseptoria tritici* (formerly known as *Mycosphaerella graminicola*, anamorph *Septoria tritici*; Quaedvlieg *et al*., 2011). The measures of disease management include fungicide sprays, the use of tolerant cultivars, and cultural practices such as crop rotation and stubble management that decrease the amount of primary inoculum (Eyal, 1999). Ascospores ejected from contaminated debris from the previous wheat crop (Sanderson, 1972) and dispersed over long distances by the wind, are the principal source of primary inoculum for *Z. tritici* infections. By contrast, pycnidiospores, which are splash-dispersed over shorter distances by the rain, are the main source of secondary inoculum during the growing period of the wheat crop (Shaw & Royle, 1989; Suffert *et al*., 2011). Furthermore, ascospores and pycnidiospores may both be involved in epidemic development, albeit at different stages (Suffert & Sache, 2011; Duvivier, 2015). In a wheat monoculture system in which contaminated debris is not completely buried, pycnidiospores can, theoretically, contribute to initiation of the epidemic (Suffert *et al*., 2011). In the spring, secondary infections may also be induced by ascospores released from old, still infectious debris in distant plots or from infected wheat plants located in the same plot (Duvivier, 2015). The level of sexual recombination in *Z. tritici* populations and the relative contributions of different forms of inoculum (pycnidiospores *vs.* ascospores; Zhan *et al.*, 1998) to the epidemic development are all the more difficult to determine experimentally since they depend on the year climatic conditions (Duvivier, 2015). Simulations with a mathematical model including the effect of spore type on epidemic development suggested that recombination can affect the genetic composition of the population during the growing season (Eriksen *et al*., 2001). In a pluriannual field experiment, Suffert & Sache (2011) and

Morais *et al*. (2016a) showed that an epidemic developed more rapidly in a plot containing ascospore-releasing debris than in a plot without debris. In the same study area, Morais *et al*. (2016b) showed how the differential adaptation of a resident population (*i.e.* originating from wheat debris of a monoculture plot) to local *vs.* distant host cultivar, can be used to infer the origin of primary inoculum. Altogether, these various observations highlight multiple and complex contributions of inoculum sources on the onset and the development of *Z. tritici* populations, suggesting that approaches complementary to classical epidemiology would be needed to disentangle the contributions of the various phenomena at work.

The spatial patterns and genotypic diversity of pathogen populations can be useful for inferring the type of inoculum playing the most important role during an epidemic period (Milgroom & Peever, 2003). Observations and modeling of the spatiotemporal dynamics of the propagules thought to act as primary inoculum can lead to the identification of sources of primary inoculum (Rieux *et al.*, 2014). Population genetics also provides information about the evolutionary processes involved in the establishment and maintenance of genetic variation within and between populations, by focusing on changes in multilocus genotypes or allele frequencies (McDonald & Linde, 2002; Abang *et al*., 2006). Population genetics studies could, therefore, be used theoretically to determine when the primary inoculum is produced and its contribution to the onset of the epidemic (Zwankhuizen *et al*., 1998; Shah *et al.*, 2001; Milgroom & Peever, 2003). In this approach, the primary inoculum may be identified as new genotypes produced through sexual reproduction and/or airborne propagules of remote origin, which may modify allele frequencies and the subsequent structure of the population. Alternatively, the primary inoculum may consist of local (*i.e.* produced in the studied area), related genotypes unlikely to have any great effect on population structure.

Empirical investigations of the genetic diversity and structure of populations of *Z. tritici* on a single date during annual epidemics have mostly been carried out at a large

geographic scale (country to continent; Zhan *et al*., 2002; 2003; Razavi & Hughes, 2004; Abrinbana *et al.*, 2010; El Chartouni *et al.*, 2011; Boukef *et al.*, 2012), with only a few rare studies focusing on local (field) or very local (plant or leaf) scales or involving assessments at multiple time points in the life cycle of the pathogen (Chen *et al*., 1994; Zhan *et al.*, 2001; Linde *et al.*, 2002; El Chartouni *et al*., 2012). Despite potential differences in the nature and amount of inoculum at different time points within an epidemic, the period of *Z. tritici* population sampling has rarely been considered as a factor potentially affecting genetic structure. Recent epidemiological studies suggest however that short-term evolution processes – including selection and counter-selection – can be driven by host cultivar adaptation and result in a trade-off between intra- and interannual scales which can be phenotypically detectable (Suffert *et al*., 2015; 2017). *Z. tritici* populations have been reported to display very high levels of genetic diversity and no structure at the continental and regional scales (Schnieder *et al.*, 2001; Linde *et al*., 2002), except in the studies of El Chartouni *et al*. (2011; 2012) and Abrinbana *et al*. (2010). Relatively little is known however about the evolution of *Z. tritici* populations over a fine spatiotemporal scale, within and among epidemic seasons. Such knowledge could be of particular interest to investigate the short-term evolutionary dynamics of *Z. tritici*, particularly the adaptation of the pathogen population (virulence and aggressiveness) to the host resistance, for instance by understanding how the different sources of inoculum can affect the genetic diversity and structure of a *Z. tritici* population during successive epidemics (Suffert *et al*., 2017). We can hypothesize that changes in the genetic diversity occurs locally at the year scale and is detectable over the course of a single annual epidemic and/or during the interepidemic period. Such a variation would be driven by variations in the relative importance of different contamination processes during epidemics. The cause would not be the "nature" of the inoculum *sensu stricto* (form of spore, pycnidiospore *vs.* ascospores) because all *Z. tritici* strains present in a field are derived from

sexual reproduction, but the "origin" of the inoculum *sensu lato*, *i.e.* its selective trajectory. At the early stages of an epidemic the initial (founder) population consisted of ascospore-derived strains having a more or less distant origin (depending on the local presence of wheat debris) and so have mainly a non-local evolutionary history. Dispersal of ascospores leads to allele pool homogenization at small spatial scales including field (Linde *et al*., 2002). Conversely, at the late stages of epidemic, the part of the population responsible for secondary infection consisted of strains having a local origin; this population is mainly derived from asexual reproduction and was potentially the result of a seasonal short-term selection driven by abiotic (fungicides sprays; temperature and host stage; Suffert *et al*., 2015) and biotic factors (host resistance; Morais *et al*. 2016b).

In this study we developed a molecular epidemiology approach to examine how the genetic structure of a *Z. tritici* population evolves in fields within and among two successive epidemics and to identify possible structuring factors. We deliberately focused on a fine spatiotemporal scale (field × cropping year) since this has never been explored in literature and since the monoculture field scale offers the possibility to avoid confounding effects (*e.g.* from the cultivars, fungicide treatments or cultural practices). We assessed and analyzed changes in the neutral genetic structure and diversity of several subpopulations collected in two close wheat fields during both epidemic and interepidemic periods of three successive years (2009-2013). The presence of wheat debris in one of the fields, yearly, enabled to contrast them for the origin of the inoculum (resident, constituted of local ascospores and pycnidiospores *vs.* distant, constituted of wind-dispersed ascospores from other fields). As it was already shown in the same study area that the local presence of inoculum had a strong quantitative effect, although transient, on the early epidemic dynamics (Suffert & Sache, 2011; Morais et *al.*, 2016b), we cannot exclude consequences on the genetic composition of the pathogen population.

Materials and methods

Experimental design and sampling

A four-year field experiment was carried out from 2009 to 2013 at the INRA Grignon experimental station (France, 48°51'N, 1°58'E), on the basis of the field design described by Suffert & Sache (2011) and Morais *et al*. (2016b). Winter wheat (cv. Soissons) was grown as a monoculture on a 20 × 100 m plot (D+) during the 2009-2010, 2010-2011, 2011-2012 and 2012-2013 cropping seasons, with no fungicide applications since 2007. The debris from the previous wheat crop was left on the soil surface and so could act as a local source of primary inoculum. Another 40 × 100 m plot (D_0; different location each year but 0-300 m distant from the D+ plot) on which wheat had not been grown in the preceding year, was planted with wheat in each of the four seasons. The primary inoculum infecting this plot was, therefore, assumed to have a distant origin. The D+ and D_0 plots were contiguous in the 2009-2010 and 2011-2012 seasons, but were located 300 m apart during the 2010-2011 and 2012-2013 season.

Sample groups originating from pycnidia (hereafter called "conidial subpopulations") were simultaneously collected from wheat leaves infected with Z. *tritici* in the D+ and D_0 plots, at one- to two-month intervals, from October 2009 to December 2012. Fungal isolates were obtained by placing the infected leaves in a plastic box with wet paper and incubating them overnight at 18°C, to promote cirrhus exudation. We removed one cirrhus per pycnidium and per leaf lesion for culture in a single Petri dish filled with PDA medium (potato dextrose agar, 39 $g.L^{-1}$) for five days. We obtained 681 pure isolates (Fig. 1). Samples collected on dates that were close together were grouped together by epidemic stage, to constitute 26 representative subpopulations of 16 to 84 individuals (P1 to P29). Consequently, four groups were studied for the 2010-2011 and 2011-2012 cropping seasons (Fig. 1):

"beginning of the epidemic period" (from October to January, corresponding to the fall and the first few weeks of winter, when the wheat was starting to tiller; subpopulations P02, P05, P09, P13, P17, P23, and P29), "intermediate epidemic period 1" (from February to late March, corresponding to the end of winter and the beginning of spring; subpopulations P10, P14, P18, and P24), "intermediate epidemic period 2" (from April to May, corresponding to the spring; subpopulations P03, P06, P11, P15, P19, and P25) and "end of the epidemic period" (from late-May to mid-July, corresponding to the end of spring and the first few weeks of summer, when the wheat reached the mature adult stage; subpopulations P04, P07, P12, P16, P21, and P27). For the 2009-2010 season, only three groups were studied due to the low disease severity in the "intermediate period", for which sampling was carried out on only one date in March 2010, the "beginning period" and the "end period" being similar, as previously described.

Sample groups originating from perithecia (hereafter called “ascospore-derived subpopulations”) were collected after the interepidemic period, from wheat debris bearing perithecia in the D+ plot. Wheat debris was kept in damp plastic boxes at 18°C overnight. The next day, the debris was cut into small pieces, each lot weighing 10 g, and spread out in fresh damp boxes (24 × 36 cm). Eight Petri dishes filled with PDA medium were placed upside down 1 cm above the debris in each box. The damp boxes were then incubated in the dark for at least 6 h at 18°C before being covered and incubated in the same conditions until the formation of colonies, which were then isolated. We obtained 115 isolates corresponding to three subpopulations (P01, P08, and P28).

The 794 pure strains used in this study were stored at -80°C in a 1:1 glycerol/water solution.

Molecular procedures

For each isolate, DNA was extracted from 50 mg of fresh fungal material scraped from a five-day culture grown on PDA medium in the dark at 18°C. DNA was extracted in an automated system (Biorobot 3000, Qiagen), with the appropriate DNeasy kit (Qiagen). All 794 isolates were genotyped for 11 single sequence repeat (SSR) markers (St2, St3A, St3C, St4, St5, St6, St7, St9, St10, St11 and St13; Gautier *et al*., 2014) in two multiplex PCRs, ensuring that amplicons with similar magnitudes of sizes bore distinct fluorochromes. Automatic allele recognition and annotation, based on binning analysis, were performed, to ensure the homogeneous naming of the alleles at each locus (Beckmann Coulter CEQ 8000 software; Gautier *et al*., 2014). In total, 585 isolates were completely genotyped for the 11 SSRs. The remaining isolates (27% of the total dataset) provided no amplicons for only one or two markers, even spread between the SSRs; they were treated as missing values in subsequent analysis. Because the proportion of identical haplotypes (clones) in the subpopulations was very low, genetic analyses were performed without clone correction (*i.e.* using only a single representative haplotype from the assessed isolates).

Mating type was determined by PCR for 739 isolates (Waalwijk *et al*., 2002). 8% of the isolates were not genotyped for mating type due to technical failures or the contamination of PCR assays.

Analyses of population subdivision without *a priori* assumptions

Population subdivision without *a priori* assumptions was investigated by the Bayesian clustering method implemented in the *STRUCTURE* program (Pritchard *et al.*, 2000). This model-based algorithm assumes linkage equilibrium within the inferred genetic clusters, and is, therefore, appropriate, in principle, for species in which recombination is a regular occurrence. We ran *STRUCTURE* with the admixture model and correlated allele frequencies. The burn-in length was set at 500,000 Markov Chain Monte-Carlo iterations. The burn-in

period was followed by a run phase of 1,000,000 iterations (El Chartouni *et al.*, 2011), with the number of clusters *K* ranging from 1 to 10, and 10 independent replicates for each value of *K*. The amount of additional information explained by increasing *K* was determined by calculating the ΔK statistic (Evanno *et al.*, 2005) with *STRUCTURE HARVESTER* (Earl & von Holdt, 2012; Belkhir *et al*., 1996-2004).

Three *STRUCTURE* analyses were carried out independently, to investigate three factors thought likely to affect the structure of the dataset: 1) collection time, during one of three or four epidemic periods during the three cropping seasons, 2) isolate origin (plot with or without debris), and 3) spore type giving rise to the isolate (ascospore or pycnidiospore). A first set of analyses was carried out to assess, for each cropping season separately (2009-2010, 2010-2011, 2011-2012), the effect of epidemic period on the structure of the pycnidiospore subpopulations collected from the plot with debris (P02-P03-P04, P09-P10-P11-P12, and P17-P18-P19-P21, respectively). An analysis grouping together the three cropping season datasets plus the data for P29 (2012-2013), was also carried out to assess the effect of year. A second set of analyses was carried out to assess, for each cropping season separately (and then overall), the effect of isolate origin (plot with or without debris) on the structure of pycnidiospore subpopulations (P02-P03-P04-P05-P06-P07, P09-P10-P11-P12-P13-P14-P15-P16, and P17-P18-P19-P21-P23-P24-P25-P27, respectively). Finally, we also analyzed the structuring effect of spore type giving rise to the isolate on the complete dataset (combining P01-P08-P28).

Analyses of population subdivision with *a priori* assumptions

Pairwise Weir & Cockerham's F_{ST} index values (Weir & Cockerham, 1984) were calculated between pairs of populations defined in Fig. 1, with *GENEPOP* V4.1 (Raymond & Rousset, 1995). The unbiased fixation index F_{ST} is widely used to assess the degree of genetic

differentiation between populations. It can be viewed as the proportion of genetic diversity due to allele frequency differences between populations or as the correlation between alleles within populations relative to the entire population. The significance of F_{ST} values was calculated after 1000 randomizations. Bonferroni correction was applied to determine the significance of *P* values.

A neighbor-joining phylogenetic tree based on distance between subpopulations was established with *POPULATIONS* (http://bioinformatics.org/~tryphon/populations/). This tree was constructed using Nei's standard genetic distance between populations (D_s, as defined in Fig. 3) and 1000 bootstraps. Finally, hierarchical analyses of molecular variance (AMOVA) were performed with *ARLEQUIN V3.5* (Excoffier & Lischer, 2010) to investigate the relative contributions of time (year and epidemic period), isolate origin and spore type giving rise to the isolate, to the partitioning of genetic variance. Each factor was tested individually with *a priori* subpopulations defined in Fig. 1 and one- or two-factor AMOVAs.

Genetic variability and occurrence of sexual reproduction

GENETIX (Belkhir *et al.*, 1996-2004) was used to estimate within-population genetic variability over time from gene diversity, calculated as multilocus observed heterozygosity (H_E), and allele richness (A_r, mean number of alleles per locus), in all populations. Paired-sample *t*-tests were used to assess the significance of differences between the D+ and D_0 plots over time (with an alpha risk of 5%).

We determine the number of unique multilocus genotypes (G) and the clonal fraction (1-G/N) with *MULTILOCUS* V1.3B (Agapow & Burt, 2001), which detects indirect evidence of sexual reproduction in *Z. tritici* populations over three cropping seasons. This software was also used to estimate multilocus linkage disequilibrium over time, by calculating the $\bar{r}_D$ index, which is corrected for the number of loci considered, and takes values from 0 (all individuals

genetically different, and multilocus linkage is broken) to 1 (all individuals are clones). The significance of the $\bar{r}_D$ values obtained was established by comparing the observed values with the distributions obtained for 1000 randomizations (Agapow & Burt, 2001). Finally, we assessed the likelihood of recombination by sexual reproduction by calculating the ratio of mating-type alleles (Mat 1-1/Mat 1-2). χ^2 tests were performed to determine whether the frequencies of the two mating types within different populations departed from the null hypothesis of a 1:1 ratio. We also performed paired-sample *t*-tests to identify significant differences over time between the D+ and D_0 plots (alpha risk of 5%) for all indices.

Results

Population partitioning

Bar plots of the three independent STRUCTURE analyses carried out to evaluate population subdivision with the admixture model and without prior assumptions about time (year and epidemic period), isolate origin (plot with or without debris), and spore type giving rise to the isolate (ascospore or pycnidiospore), are provided in Fig. 2. The three cropping seasons (2009-2010, 2010-2011, 2011-2012) and the adjacent epidemic periods (end of the 2008-2009 cropping season, P01; start of the 2012-2013 cropping season, P29) are presented together. In the first set of analyses, pycnidiospore subpopulations from the D+ plot were analyzed for each cropping system separately (Supplementary Table 1) and then for all cropping seasons together (Supplementary Table 1; Fig. 2A). The rate of change in the log probability of data between successive *K* values (ΔK) had a mode at $K = 3$ for the 2009-2010 season, at $K = 2$ for the 2010-2011 and 2011-2012 cropping seasons, and at $K=7$ for the overall dataset. Regardless of the epidemic period considered in each cropping season (year), it was difficult to assign individuals to any one of these *K* clusters (none of the individuals were assigned to one cluster with a posterior probability > 0.5). Instead, the posterior probability was split

equally between the seven clusters (0.1426 < overall mean P < 0.1431), suggesting that neither the epidemic period nor the cropping season had any major effect on the structure of pycnidiospore subpopulations. In the second set of analyses, pycnidiospore subpopulations from the D+ and D_0 plots were analyzed for each cropping season separately (Supplementary Table 1) and then for all cropping seasons together (Supplementary Table 1; Fig. 2B). Modes were observed at K = 3 for the 2009-2010 season and at K = 2 for the 2010-2011, and 2011-2012 cropping seasons and for the overall dataset. The posterior probability of individuals was equally distributed between the two clusters (0.498 < overall mean P < 0.502). Only 0.15 % of the individuals were assigned to one cluster with a posterior probability greater than 0.6, whatever the epidemic period, again suggesting that isolate origin (D+ or D_0 plots) had little effect on the structure of the dataset. Finally, after the addition of ascospore subpopulations to the previous dataset in the third *STRUCTURE* analysis (Supplementary Table 1; Fig. 2C), a mode at K = 3 and evenly distributed posterior probabilities (0.332 < overall mean P < 0.334) were again observed. We can therefore conclude that spore type, giving rise to the isolate, had little effect on structure.

As no population structure was detected without prior assumptions, we explored the dataset with the *a priori* subpopulations defined in Fig. 1. The relationships between the 26 subpopulations are presented in the phylogenetic tree (Fig. 3), on which it is difficult to distinguish the various subpopulations due to the low bootstrap values for the nodes. Nevertheless, some subpopulations were localized to longer branches of the tree: P13, P16, P25 and P27 (pycnidiospore subpopulations) and P08 (ascospore subpopulation).

We investigated the differentiation within and between subpopulations obtained from pycnidiospores and ascospores, by calculating the pairwise F_{ST} . The overall mean F_{ST} was 0.016, with 22.5% of individual F_{ST} values significant before Bonferroni correction, and 1.2%

significant after this correction. Significant F_{ST} values were obtained for populations P16, P17, P24 and P25 (Supplementary Table 2).

We then examined the partitioning of genetic variation between the various factors potentially shaping the structure of *Z. tritici* populations (year and epidemic period, isolate origin, spore type), through hierarchical analyses of molecular variance (AMOVA) (Tables 1 and 2; Supplementary Table 3).

A first two-factor AMOVA, assessing the effect of year (cropping season) and epidemic period nested within cropping season in each plot separately indicated that variation within epidemic periods accounted for most of the molecular variance (98-99%; $P < 0.05$; $0.012 < F_{ST} < 0.020$). Variation between epidemic periods within cropping seasons accounted for only 1.7-2.1% of the molecular variance, but was nevertheless significant ($P \leq 0.05$). Thus, the genetic variation between subpopulations was generally little affected by the epidemic period. We then performed a set of one-factor AMOVAs, to investigate the effect of isolate origin nested within epidemic period (Table 2). We found that, for all epidemic periods, within-plot variation accounted for most of the molecular variance (97.6-99.6%). Nevertheless, there was significant variation between plots for intermediate epidemic periods 1 and 2, even though this variation explained only a very small proportion of the molecular variance (1.9-2.4%; $P < 0.034$; $0.019 < F_{ST} < 0.024$). The genetic variation of the populations was therefore slightly, but significantly, affected by isolate origin (plot D+ or D_0), exclusively between January and April, when the number of airborne ascospores is generally lower than at the beginning and end of epidemics. A third set of AMOVA analyses (Table S3) was performed to assess the effect of spore type giving rise to the isolate on the genetic structure of subpopulations at relevant epidemic periods (end/beginning) and to test the hypothesis of temporal continuity. We compared genetic variation between the pycnidiospore subpopulations collected at the end of each epidemic (P04, P21) and the ascospore

subpopulations present on the debris of the corresponding crop collected at the start of the next epidemic (P08, P28, respectively) in a D+ plot, and then in a D_0 plot (P07, P27, respectively; Table S3A). Within-plot variation accounted for most of the molecular variance (96.7-100%). Between-plot variation was not significant, except for the P27-P28 comparison (3.4%; $P < 0.05$; $F_{ST} = 0.033$); the difference in genetic structure between P27 and P28 was therefore small, but significant. We tested the hypothesis that early infections in D+ plots were caused principally by local inoculum resulting from a build-up of ascospores ejected from the debris of the previous wheat crop. Each ascospore population collected from wheat debris at the beginning of an epidemic (P01, P08, P28) was compared with the pycnidiospore subpopulations collected during the same period from D+ plots (P02, P09, P29, respectively), and from D_0 plots (P05, P13; Table S3B). In all cases, within-population variation accounted for most of the molecular variance (89.2-100%). The differences between ascospore and pycnidiospore subpopulations were small and non-significant for both D+ plots (-0.4-3.4%; $0.116 < P < 0.659$; $-0.004 < F_{ST} < 0.033$) and for D_0 plots (0.57% and 10.1%; $0.118 < P < 0.279$; $0.006 < F_{ST} < 0.101$). This lack of difference in the genetic structure of the populations makes it impossible to determine, for D+ plots, whether the primary inoculum was of local or distant origin (*i.e.* originating from resident or immigrant populations).

Changes in population diversity over the three successive cropping seasons

The genetic variability within each of the 26 *Z. tritici* populations (Fig. 1) was the first estimated by calculating genic non-biased heterozygosity diversity, H_E (Fig. 4A). H_E values were of the same order of magnitude for all populations and varied between 0.38 and 0.58, with a mean at 0.46, 0.44 and 0.42 for the pycnidiospore populations from D+ and D_0 plots and for ascospore populations, respectively. Finally, we found no significant difference in the

evolution of populations from the D+ and D_0 plots, in a *t*-test for paired populations (P = 0.471).

The mean number of alleles per locus (allele richness A_r) ranged between 2 and 6.27 (mean 4.45, 3.67 and 4.06 for the D+, D_0, and ascospore populations, respectively) for the three cropping seasons considered (Fig. 4B). The highest A_r values were found for the 2011-2012 cropping season, which was also the season in which the number of strains collected was largest. Allele richness in the D+ plots was at least as high as that in D_0 plots, except for the second intermediate period of the 2011-2012 cropping season, a finding not consistent with a difference in population size. No significant difference was observed between the D+ and D_0 populations over time (P = 0.084).

Occurrence of sexual reproduction in the field population of *Z. tritici*

The two mating types, Mat1-1 and Mat1-2, were found in all subpopulations (Fig. 5A) other than P13, a D_0 pycnidiospore subpopulation consisting of only five individuals of the Mat1-2 type. The Mat1-1/Mat1-2 mating-type ratio calculated for all subpopulations, including only isolates with a complete mating type (703 of the 794 isolates), was 0.95. In χ^2 tests, the frequency of the two mating types was not significantly different from the expected 1:1 ratio (P = 0.126).

We then assessed the difference in mating-type ratio between plots. The mean ratio was 1.22 for the D+ pycnidiospore subpopulations and 0.71 for the D_0 pycnidiospore subpopulations (Fig. 5A). In χ^2 tests, the ratio of mating type frequencies within each plot did not differ significantly from 1:1 (P = 0.407 for D+; P = 0.746 for D_0). However, a *t*-test for paired samples revealed a significant difference in this ratio between the two plots over time (P = 0.008).

Finally, we assessed differences in mating-type ratio between epidemic periods. In χ^2 tests, the ratio of the two mating types in pycnidiospore subpopulations collected at the “end” of epidemic periods was significantly different from 1:1 (mean ratio = 1.26; P = 0.047), whereas no such difference was observed at the "beginning" of epidemics (P = 0.687).

Linkage disequilibrium was always low (close to full panmixia), with $\bar{r}_D$ ranging from negative values to a maximum of 0.09 (P16), with a mean value of 0.006, 0.015, and 0.001 for the D+, D_0, and ascospore subpopulations, respectively (Fig. 5B). Again, no significant differences were found between the D+ and D_0 plots (P = 0.354), despite $\bar{r}_D$ being greater at the beginning and end of the epidemic periods of the 2010-2011 cropping season in D_0 plots.

The clonal fraction was very low over the three cropping seasons (Fig. 5C): with mean values of 0.06 for pycnidiospore subpopulations from both D+ and D_0 plots, and 0.03 for ascospore subpopulations, with values ranging from a minimum of 0 (no clone found in the subpopulation) to a maximum of 0.2 (only two clones in the five-isolate subpopulation, P13). No significant difference in clonal fraction was found between the two plots (P = 0.784).

Discussion

In this study, we aimed to identify neutral structure over the course of three successive annual epidemics at the field scale. Indeed, various inoculum sources, local or distant, may contribute to the onset and/or the development of the epidemics, with different and year-dependent timings. We hypothesized that either 1) the yearly variation in population size over epidemic courses and the regular introduction of distant “original” or “new” genotypes may induce variations in population diversity at the scale of the epidemiological cycle, or, alternatively, 2) that the demographic fluctuations endured by local populations had little effect on their diversity, with respect to the high diversity described at larger spatial scales.

We initially used an assumption-free approach for individual assignment and the testing of three putative structuring factors: time period, plot of origin and spore type giving rise to the isolate. We found no relevant neutral population structure due to any of these factors. In particular, there were no differences in the genetic structure of subpopulations collected early and late in the same season, or between the monthly samples collected from the same plot over a three-year period. These findings are consistent with the low level of between-population differentiation and the weak support for population locations on the phylogenetic tree. These observations are also consistent with previous results showing that the genetic structure of *Z. tritici* populations was stable over time, for annual, three-year (Chen *et al*., 1994) and six-year collections (Zhan *et al*., 2001), and in space, over spatial scales ranging from meters to thousands of kilometers (Linde *et al*., 2002; Schnieder *et al*., 2001), even if variations in nested smaller scales had not been checked. Accordingly, the 794 isolates collected locally constituted a single, highly diverse entity that remained stable over all epidemic stages, regardless of the demographic features of the population. This panmictic population, large and unstructured, did not appear to be affected by the putative origin of the inoculum colonizing the plots, at least in the sampled years and, in particular, the introduction of wind-dispersed distant inoculum had no measurable effect. The airborne inoculum mainly consist of local genotypes resulting from sexual reproduction in the wheat debris of the plots studied (at least during the early stage of epidemics), but it probably contained several genotypes of distant origin, given the long-distance dispersal capacities of *Z. tritici* ascospores. Indeed, the plot without debris (D_0) was recolonized each year by inoculum of unknown distant origin, whereas the plot with debris (D+) was recolonized mostly by local inoculum, as showed by previous results obtained from comparisons of aggressiveness and the host adaptation of local strains (Morais *et al*., 2016a; 2016b) in the same experimental study area. The lack of genetic structure, resulting in similar frequencies of neutral alleles in

the different subpopulations, did not allow to confirm the partially distinct origin of inoculum in the two plots which was inferred from differential adaptation to wheat cultivars established by phenotyping (Morais *et al*., 2016b). Results obtained in the current study are however not inconsistent with the previous because SSRs are not "filtered" (*i.e.* selected) by the wheat cultivar and are so no relevant to highlight clean-cut patterns of host or local adaptation.

Nevertheless, the analysis of molecular variance (AMOVA) of populations defined *a priori* on the basis of our sampling scheme (Fig. 1) revealed that sampling time, particularly during the epidemic period, could have a slight (< 2% of molecular variance) but significant effect on the differentiation of conidial subpopulations in the two plots studied. Similarly, plot of origin explained a small (< 2.5%) even if significant proportion of the molecular variance during the growing season (intermediate periods 1 and 2, i.e. between February and May, when *Z. tritici* subpopulations are thought to expand through asexual reproduction). Finally, we found that molecular variance was similar between the conidial and ascospore-derived subpopulations collected at the end of the cropping season, with one exception (Table S3): there was weak, but significant differentiation between the conidial subpopulation from the plot without debris at the end of epidemic period (P27; season 2011-2012) and the ascospore-derived population from the plot with debris collected at the beginning of the next season (P28; 2012-2013). This effect was not detected in previous studies of single strains collected yearly (Chen *et al.*, 1994; Zhan *et al.*, 2001). The differences could be an artefact, due for instance to the sensitivity level of the AMOVA test used or to the sample size. While number of isolates was small in some subpopulations due to field technical constraints, the average size of each subpopulation was 30 and the total number of isolates was 794, which is correct to detect overall differentiation among populations - if exists - by using 11 SSRs.

Genetic diversity was high to very high in all subpopulations, and stable over the three cropping seasons (variation over time non-significant). The orders of magnitude of expected

heterozygosity (H_E), allele richness (A_r) and the proportion of multilocus haplotypes were consistent, although somewhat smaller, with the values obtained with SSRs over larger geographic scales for Israel, Swiss, USA, Tunisian, French and Canadian populations (Linde *et al*., 2002; El Chartouni *et al*., 2011; Boukef *et al*., 2012; Razavi & Hughes, 2004). Conidial and ascospore-derived subpopulations did not differ in diversity, whereas higher levels of genotypic diversity might have been expected after sexual recombination, which is thought to occur in winter. No significant difference in the evolution of diversity was found between the two plots, so the larger amount of local primary inoculum produced in the plots containing debris had little or no effect on diversity. This contrasts with the selection thought to be exerted on some loci during the epidemic period by cultivar (Morais *et al*., 2016b), temperature (Suffert *et al.*, 2015), or fungicides (Hayes *et al*., 2016) or with the putative counter-selection during the interepidemic period (Suffert *et al*., 2017). Our findings are not inconsistent with such selective dynamics as we focused actually on neutral diversity. Population differentiation at quantitative, adaptive traits is not directly linked with neutral genetic diversity or differentiation; even if neutral genetic markers have great potential for investigating processes such as gene flow, migration or dispersal, this type of genetic variation tells us nothing about the adaptive or evolutionary potential of a population (Holderegger *et al*., 2006). Some selected markers could be developed from recently described genes determining *Z. tritici* adaptation, for instance adaptation to the Stb6 resistance gene present in many French cultivars (Zhong *et al*., 2017) or to DMI fungicides largely used in Western Europe (Cools *et al*., 2013), and would help to clarify this issue. Such selected markers would be particularly relevant since the genes in the pathogen population are not yet widespread (*e.g.* range from 5 % to 25 %) and not homogeneously distributed at a small spatial scale; this may be the case for a virulence corresponding to a resistance very recently overcome or the case of a fungicide resistance recently appeared.

The ratio of the two mating types did not differ significantly from the expected 1:1 ratio, consistent with previous results (Zhan *et al.*, 2002; Siah *et al*., 2010). The presence of both idiomorphs, Mat 1-1 and Mat 1-2, in all populations, albeit in variable proportions, confirmed that sexual reproduction could occur all year round, although this might require specific climatic conditions and host tissue physiological status. A χ^2 test on mating-type frequencies at the beginning and end of the three epidemic periods (not significantly different from 50% versus significantly higher than 50%) revealed a slight increase in the frequency of Mat 1-1 isolates over the cropping system. This may reflect the higher pathogenicity of Mat 1-1 isolates than of Mat 1-2 isolates (Zhan *et al*., 2007), which should be explored *in planta,* with our isolates. Linkage disequilibrium remained very low over time, at values close to full panmixia, regardless of primary inoculum origin. Similarly, the clonal fraction was always low, regardless of sampling time and inoculum origin. For example, the 2009-2010 cropping season was characterized by more intense ascospore production in the plot with debris than during the other two cropping seasons studied (Morais *et al*., 2016a), but this had little effect on the patterns of diversity indices over the cropping season. This is not surprising to find clones in ascospore-derived populations, because four pairs of haplotypes occur in each single ascus (Kema *et al*., 1996) and could be isolated by chance. Overall, these results are consistent with recombination occurring regularly, due to sexual reproduction over a long period of the year, but impossible to pin down with these data, which are, again, of a similar order of magnitude to data already reported for this fungal species at a larger geographic scale. This suggests that local stubble management, while decreasing the local production of ascospores and modifying the amount of local primary inoculum, may not be sufficient to induce local bottlenecks, instead being compensated by intense migration. This conclusion should be however confirmed for traits subject to selection using relevant markers.

Finally, local *Z. tritici* populations displayed high levels of genetic uniformity between years, epidemic periods and plots, which consistent with the results obtained by Chen *et al*. (1994), Zhan *et al.* (2002) and El Chartouni *et al*. (2012) but observed at a fine temporal scale, not studied before. Our demogenetic analysis of a local population thought to result from different evolution trajectories through three successive sequences of epidemic and interepidemic periods highlights only sporadic and small effects of the type of inoculum, depending on epidemic period and year. Analogous hypotheses about the respective contributions of sexual/asexual primary/secondary inoculum were tested by Cortesi & Milgroom (2001) and Shah *et al*. (2001), who investigated spatial patterns and genetic diversity. Such studies, which remain uncommon, especially at the fine spatiotemporal scale that we used, show how population genetics can be used to address epidemiological questions essential for sound disease management (Milgroom & Peever, 2003) but also what are its limits and opportunities for further improvement.

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Figures

Figure 1: Subpopulations of *Z. tritici* (794 isolates) collected from two wheat plots (D+, with debris; D_0, without debris) over the course of three successive annual epidemics (2009-2010, 2010-2011 and 2011-2012) in Thiverval-Grignon, France.

The 794 isolates were grouped according to the epidemic period (beginning, i.e. from late-November to late-January; intermediate 1, i.e. February and March; intermediate 2, i.e. from April and May; end, i.e. from early-June to mid-July). White columns: pycnidiospore subpopulations from the plot with debris (D+); gray columns: pycnidiospore subpopulations from the plot without debris (D_0); black columns: ascospore subpopulations from the plot with debris (D+).

Figure 2: Genetic subdivision of the 794 *Z. tritici* isolates collected over the course of three successive annual epidemics (2009-2010, 2010-2011 and 2011-2012) in Thiverval-Grignon, France.

Colors indicate the membership of *K* clusters for the individuals, these clusters being as defined after STRUCTURE analysis, to test three structure hypothesis. A - Effect of epidemic period (*K* = 7; 460 isolates; letters indicate the different epidemic periods (B: beginning; I1 and I2: intermediate; E: end). B - Effect of isolate origin (*K* = 2; 685 isolates; D+: plot with debris, D_0: plot without debris). C - Spore type giving rise to the isolate (*K* = 3; 794 isolates; A: ascospores; P: pycnidiospores). For each barplot, the vertical black lines delimit the different cropping seasons and the vertical white lines delimit the second factor studied (epidemic period in A; isolate origin in B; spore type in C).

Figure 3: Phylogenetic tree based on molecular distance between the 26 *Z. tritici* populations (794 isolates; see Fig. 1) collected over the course of three successive annual epidemics (2009-2010, 2010-2011 and 2011-2012).

Tree established by the neighbor-joining method, based on Nei's standard genetic distance (Ds), with 1000 bootstraps, on loci.

Figure 4: Temporal changes in the diversity of 26 *Z. tritici* populations (794 isolates; see Fig. 1) collected from wheat plots with (D+) and without (D_0) debris, over the course of three successive annual epidemics (2009-2010, 2010-2011 and 2011-2012).

A - Observed heterozygosity (H_E). B - Allele richness (A_r, mean number of alleles per locus). White circles: pycnidiospore subpopulations from D+ plots; black diamonds: pycnidiospore subpopulations from D_0 plots; crosses: ascospore subpopulations.

Figure 5: Evidence of sexual reproduction in 26 *Z. tritici* subpopulations (794 isolates; see Fig. 1) collected from wheat plots with (D+) and without (D_0) debris, over the course of three successive annual epidemics (2009-2010, 2010-2011 and 2011-2012).

A - Changes in mating-type ratio (Mat1-1/Mat1-2) over time in pycnidiospore and ascospore subpopulations. B - Changes over time in linkage disequilibrium, estimated by calculating the r_{D^-} index. C - Changes over time in the clonal fraction (1-G/N) on the same plots. Symbols indicate the origin of the populations: white circles: pycnidiospore subpopulations from D+ plots; black diamonds: pycnidiospore subpopulations from D_0 plots; crosses: ascospore subpopulation.

Table 1: Hierarchical analysis of molecular variance (AMOVA) with cropping season and epidemic period nested within cropping season as grouping factors, for the D+ (upper panel) and D_0 (lower panel) plots.

	df	Sum of squares	Variance components	% variation	*P*-value	Fixation index
Plots with debris (D+)						
Between cropping seasons	2	3.6	-0.01	-0.56	0.935	-0.005
Between epidemic periods Within cropping seasons	8	23.6	0.032	1.71	**0.001**	0.017
Within epidemic periods	386	714.6	1.851	98.85	**< 0.001**	0.012
Total	*396*	*741.7*	*1.873*			
Plots without debris (D_0)						
Between cropping seasons	2	4.7	-0.0003	-0.02	0.349	< 0.001
Between epidemic periods Within cropping seasons	8	18.2	0.034	2.05	**0.005**	0.020
Within epidemic periods	210	341.6	1.626	97.97	**0.002**	0.020

Total	*220*	*364.5*	*1.660*

Table 2: Hierarchical analysis of molecular variance (AMOVA), with the collection plot (D+ or D_0) and isolate origin as grouping factors, for each epidemic period

	df	Sum of squares	Variance components	% variation	*P*-value	Fixation index
Beginning of epidemics						
Between plots	1	2.4	0.007	0.37	0.183	0.003
Within plots	193	370.4	1.919	99.63		
Total	*194*	*372.8*	*1.926*			
Intermediate period 1						
Between plots	1	2.8	0.029	1.92	**0.034**	0.019
Within plots	89	133.3	1.498	98.08		
Total	*90*	*136.1*	*1.777*			
Intermediate period 2						
Between plots	1	4.3	0.038	2.37	**0.002**	0.024
Within plots	145	225.7	1.556	97.63		

Total	*146*	*230.0*	*1.594*			
End of the epidemics						
Between plots	1	2.8	0.014	0.80	0.074	0.008
Within plots	183	312.6	1.708	99.20		
Total	*184*	*315.3*	*1.722*			

FIGURE1

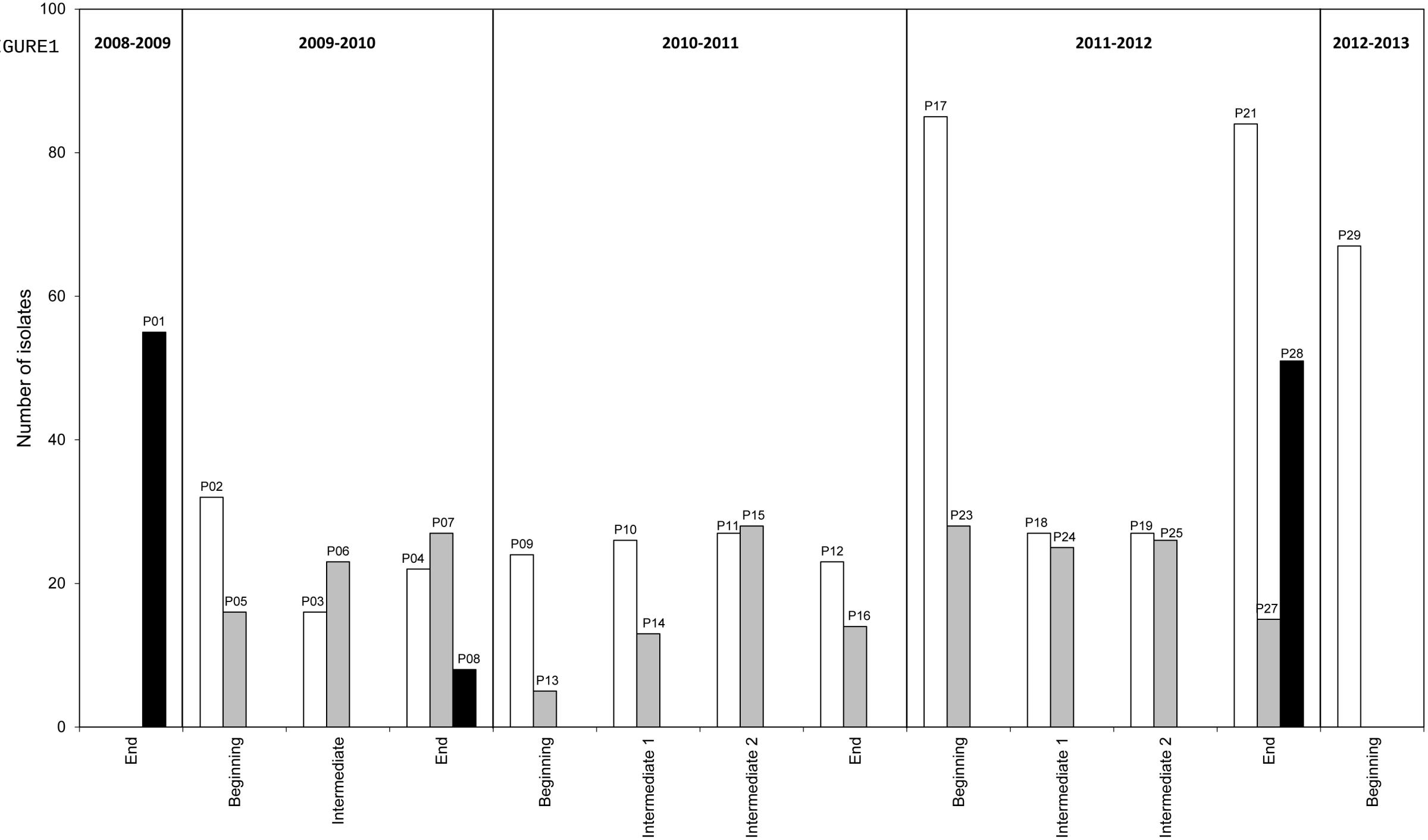
2008-2009
2009-2010
2010-2011
2011-2012
2012-2013
Number of isolates
100
80
60
40
20
0
P01
P02
P05
P03
P06
P04
P07
P08
P09
P13
P10
P14
P11
P15
P12
P16
P17
P23
P18
P24
P19
P25
P21
P27
P28
P29
End
Beginning
Intermediate
End
Beginning
Intermediate 1
Intermediate 2
End
Beginning
Intermediate 1
Intermediate 2
End
Beginning

FIGURE2

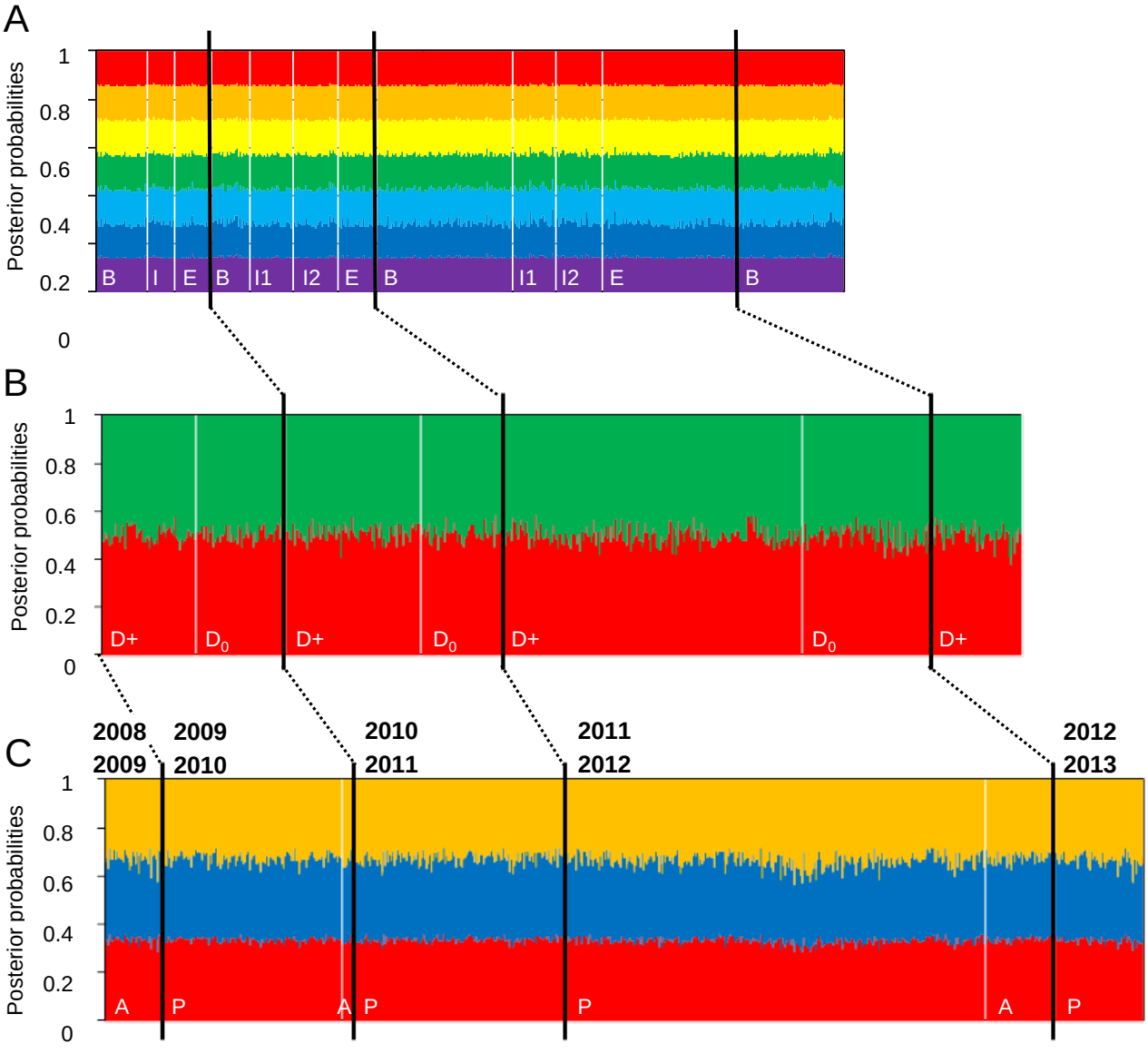
A
Posterior probabilities
1
0.8
0.6
0.4
0.2
0
B
I
E
B
I1
I2
E
B
I1
I2
E
B
B
Posterior probabilities
1
0.8
0.6
0.4
0.2
0
D+
D0
D+
D0
D+
D0
D+
C
2008
2009
2009
2010
2010
2011
2011
2012
2012
2013
Posterior probabilities
1
0.8
0.6
0.4
0.2
0
A
P
A
P
P
A
P

FIGURE3

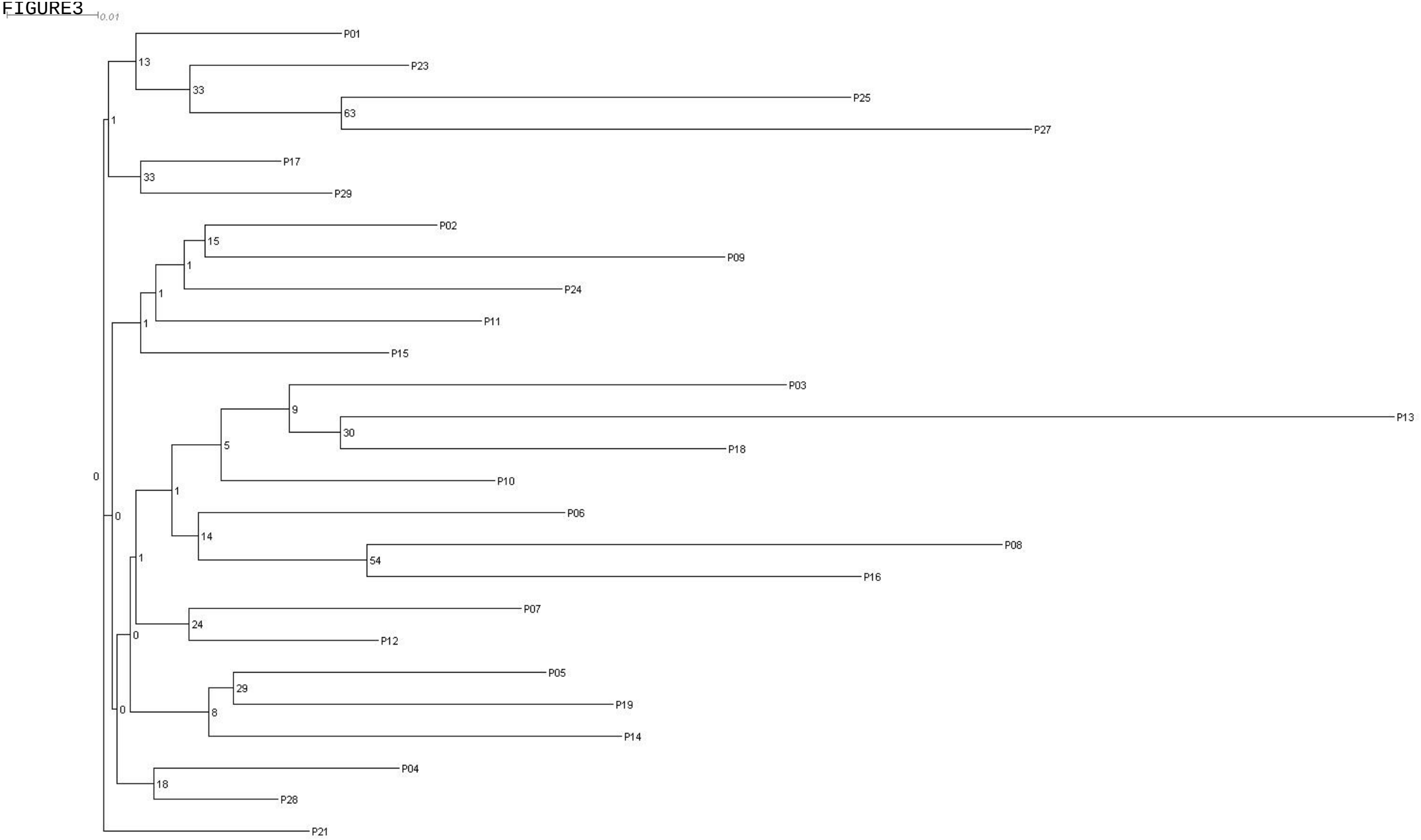
0.01
P01
13
P23
33
P25
63
P27
1
P17
33
P29
P02
15
P09
1
P24
1
P11
1
P15
P03
9
P13
30
P18
5
P10
0
1
P06
14
P08
54
P16
1
P07
24
P12
0
P05
29
P19
0
8
P14
P04
18
P28
P21

FIGURE4

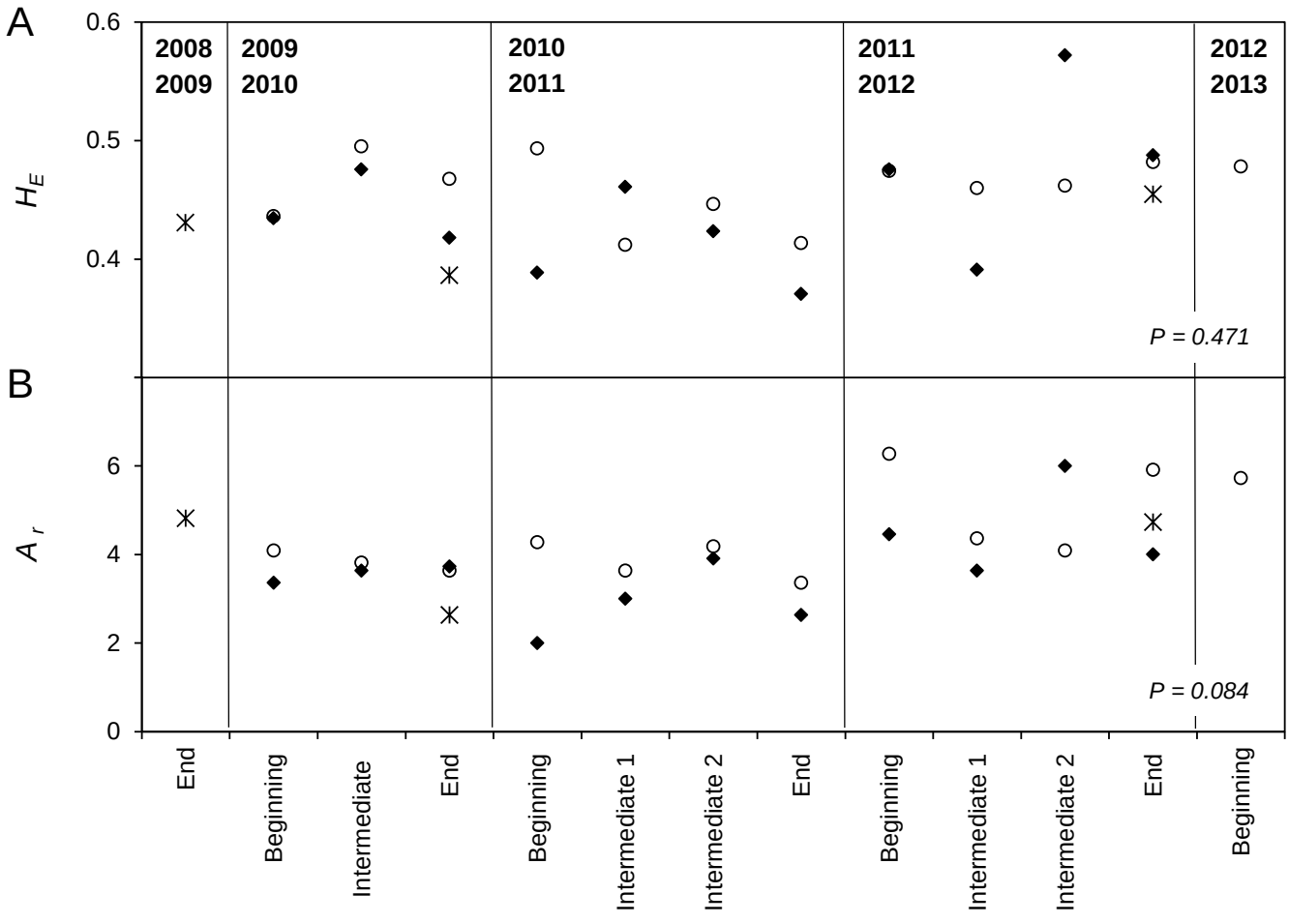
A
B
H_E
A_r
0.6
0.5
0.4
6
4
2
0
2008
2009
2009
2010
2010
2011
2011
2012
2012
2013
P = 0.471
P = 0.084
End
Beginning
Intermediate
End
Beginning
Intermediate 1
Intermediate 2
End
Beginning
Intermediate 1
Intermediate 2
End
Beginning

FIGURE5

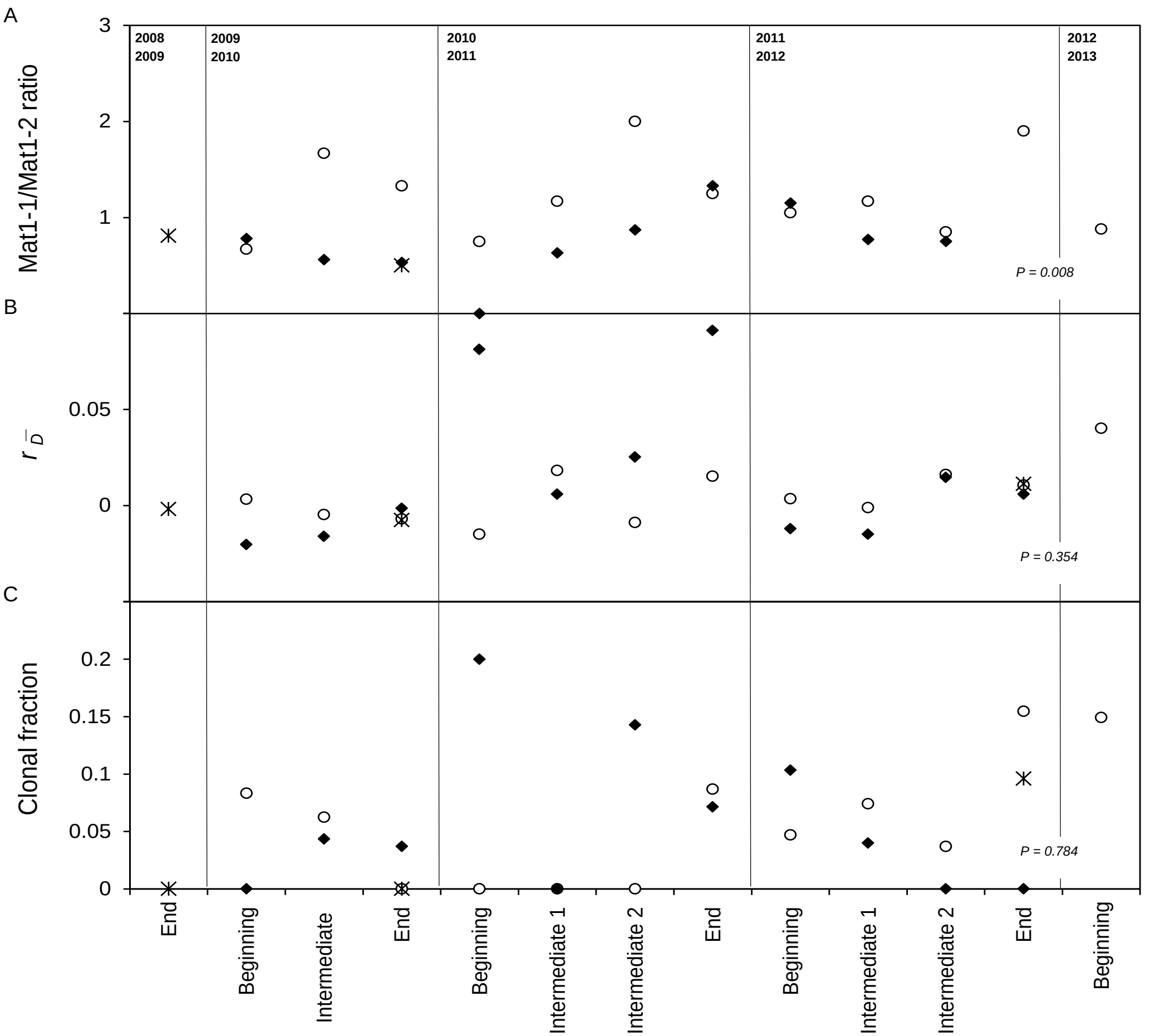
A
B
C
Mat1-1/Mat1-2 ratio
$r_{\bar{D}}$
Clonal fraction
2008
2009
2009
2010
2010
2011
2011
2012
2012
2013
P = 0.008
P = 0.354
P = 0.784
3
2
1
0.05
0
0.2
0.15
0.1
0.05
0
End
Beginning
Intermediate
End
Beginning
Intermediate 1
Intermediate 2
End
Beginning
Intermediate 1
Intermediate 2
End
Beginning

Table S1: Results of the clustering analysis performed with STRUCTURE for isolates collected over the course of three successive annual epidemics (2009-2010, 2010-2011 and 2011-2012) and genotyped with 11 SSR markers (Gautier *et al*., 2014).

All runs were replicated 10 times (burn-in period = 500000 and MCMC iterations = 1000000), for each cropping season separately and for all cropping seasons together. The optimal number of clusters *K* (Evanno *et al*., 2005) is shown in bold; clustering corresponding to the underlined values is presented in Fig. 2.

A. Effect on epidemic period with pycnidiospore subpopulations from the D+ plot (460 isolates)

	2009-2010		2010-2011		2011-2012		Overall	
K	|Ln"(*K*)|	Delta *K*	|Ln"(K)|	Delta *K*	|Ln"(K)|	Delta *K*	|Ln"(*K*)|	Delta *K*
1	—	—	—	—	—	—	—	—
2	6.810	1.981	**16.780**	**14.376**	**170.100**	**20.254**	7.310	2.277
3	**17.170**	**2.310**	1.850	0.526	1226.580	13.177	83.810	3.797
4	2.200	0.144	6.720	0.573	870.310	2.938	138.920	2.860
5	6.270	0.853	33.250	1.682	1516.770	1.965	115.270	1.050
6	7.040	0.551	5.740	0.314	803.630	2.334	417.540	1.169
7	4.300	0.523	20.250	1.144	162.970	0.864	**3314.540**	**13.881**
8	1.290	0.146	18.130	0.662	78.210	0.705	860.940	0.497
9	2.120	0.223	0.270	0.012	39.590	0.567	3841.650	1.941
10	—	—	—	—	—	—	—	—

B. Effect of isolate origin (D+ and D_0 plots) for pycnidiospore subpopulations (681 isolates)

	2009-2010		2010-2011		2011-2012		Overall	
K	|Ln"(*K*)|	Delta *K*	|Ln"(*K*)|	Delta *K*	|Ln"(*K*)|	Delta *K*	|Ln"(*K*)|	Delta *K*

1	—	—	—	—	—	—	—	—
2	4.920	2.128	**18.490**	**17.034**	**202.800**	**27.063**	**1396.743**	**17.451**
3	**15.600**	**3.907**	7.950	1.716	836.650	16.871	1782.690	6.325
4	7.570	0.820	6.020	0.515	445.660	1.656	779.260	1.771
5	4.740	0.317	2.830	0.166	139.360	0.508	311.320	0.847
6	23.550	1.183	26.720	1.501	1100.370	0.913	1203.560	3.404
7	56.210	1.291	26.470	1.783	368.040	0.833	476.760	3.336
8	35.720	1.227	26.980	0.814	258.460	0.170	363.870	0.434
9	21.550	0.790	20.320	1.408	99.900	0.071	907.120	1.504
10	—	—	—	—	—	—	—	—

C. Effect of spore type giving rise to the isolate (ascospore or pycnidiospore) for subpopulations from the D+ and D_0 plots (800 isolates)

	Overall	
K	|Ln"(*K*)|	Delta *K*
1	—	—
2	133.443	15.668
3	**1031.870**	**18.864**
4	138.800	0.497
5	727.010	2.641
6	168.030	0.268
7	1155.840	2.663
8	1298.330	2.022
9	654.750	1.076
10	—	—

Table S2: Pairwise F_{ST} between the 26 *Z. tritici* subpopulations (see Fig. 1) collected in Thiverval-Grignon (France).

Subopulations are classified according to spore type giving rise to the isolate (pycnidiospore, ascospore), cropping season (2009-2010, 2010-2011 and 2011-2012), epidemic period (beginning, intermediate 1, intermediate 2, end) and origin (D+ plot, D_0 plot). Bold values highlighted in yellow indicate F_{ST} with $P < 0.05$ (significant); only bold values highlighted in red are still significant after the Bonferroni correction.

Type of spores					pycnidiospores																								ascospores	
	Cultural season				2009-10						2010-11								2011-12								2012-13	2008-09	2009-10
		Epidemic period			Beginning		Intermediate		End		Beginning		Intermediate 1		Intermediate 2		End		Beginning		Intermediate 1		Intermediate 2		End		Beginning	End	
			Plot		D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D_0	D+	D+	
				Pop	P02	P05	P03	P06	P04	P07	P09	P13	P10	P14	P11	P15	P12	P16	P17	P23	P18	P24	P19	P25	P21	P27	P29	P01	P08
pycnidiospores	2009-10	Beginning	D_0	P05	0.002																								
		Intermediate	D+	P03	0.017	0.009																							
			D_0	P06	**0.029**	0.019	-0.008																						
		End	D+	P04	0.004	-0.011	0.022	0.016																					
			D_0	P07	0.012	0.025	0.021	0.001	0.008																				
	2010-11	Beginning	D+	P09	0.008	-0.012	0.018	0.020	0.006	0.012																			
			D_0	P13	0.042	0.020	-0.020	-0.007	0.034	0.018	0.007																		
		Intermediate 1	D+	P10	0.010	0.021	-0.008	0.011	0.024	0.005	**0.026**	0.040																	
			D_0	P14	-0.001	-0.011	0.016	0.039	0.009	0.025	-0.002	-0.011	0.028																
		Intermediate 2	D+	P11	-0.006	0.023	0.013	0.020	0.017	0.006	0.003	0.051	0.000	0.000															
			D_0	P15	0.012	0.019	**0.034**	0.004	0.001	-0.009	0.016	0.060	0.005	0.026	0.013														
		End	D+	P12	0.008	-0.020	0.001	-0.004	-0.010	-0.008	0.005	0.027	-0.006	0.025	0.018	-0.010													
			D_0	P16	**0.060**	**0.061**	0.044	0.019	**0.082**	**0.037**	**0.041**	0.078	0.018	0.039	0.027	0.021	**0.043**												
	2011-12	Beginning	D+	P17	0.005	0.002	0.000	0.009	0.008	0.002	0.005	0.029	0.005	0.011	-0.007	**0.015**	0.000	**0.033**											
			D_0	P23	-0.007	-0.003	-0.008	0.000	-0.007	-0.001	0.011	-0.008	0.008	0.013	0.009	0.003	-0.010	**0.059**	0.002										
		Intermediate 1	D+	P18	**0.023**	**0.030**	0.010	**0.033**	**0.028**	0.014	**0.037**	0.016	0.010	0.011	0.011	**0.036**	0.024	0.031	0.012	**0.026**									
			D_0	P24	0.007	0.003	0.027	**0.027**	-0.009	0.006	0.021	0.046	0.018	0.036	0.018	0.012	-0.012	**0.096**	**0.015**	-0.002	**0.027**								
		Intermediate 2	D+	P19	**0.034**	0.026	**0.041**	**0.035**	**0.037**	0.008	0.022	-0.013	**0.029**	-0.008	0.018	**0.037**	**0.033**	**0.049**	**0.023**	0.021	**0.029**	**0.049**							
			D_0	P25	**0.023**	0.008	0.014	**0.038**	0.009	**0.039**	**0.033**	0.017	**0.042**	0.019	**0.038**	**0.041**	0.018	**0.090**	**0.030**	0.007	**0.026**	0.018	**0.051**						
		End	D+	P21	**0.016**	-0.005	0.008	0.011	0.004	0.005	**0.018**	0.026	0.003	0.015	**0.018**	0.000	-0.011	**0.035**	**0.008**	0.006	**0.018**	0.014	**0.030**	**0.023**					
			D_0	P27	0.026	**0.045**	0.027	**0.057**	**0.040**	0.016	**0.034**	0.017	**0.042**	0.000	**0.028**	**0.040**	0.032	**0.065**	**0.035**	0.001	**0.037**	**0.042**	0.024	0.009	**0.033**				
	2012-13	Beginning	D+	P29	-0.001	0.018	-0.003	0.006	0.011	-0.002	0.018	0.011	-0.004	0.002	-0.001	0.000	0.006	0.022	0.004	-0.004	0.009	**0.017**	**0.023**	**0.035**	**0.010**	0.019			
ascospores	2008-09	End	D+	P01	-0.002	-0.001	**0.023**	**0.025**	0.005	0.013	0.017	0.064	0.006	0.013	0.005	0.007	-0.003	**0.048**	0.007	0.004	**0.025**	0.010	**0.037**	**0.028**	**0.008**	**0.037**	0.004		
	2009-10			P08	0.023	0.000	0.012	0.009	0.011	-0.001	0.014	0.069	-0.023	0.011	0.015	-0.029	-0.019	-0.007	0.001	0.006	0.024	0.025	0.018	0.027	-0.017	0.021	-0.002	-0.008	
	2011-12			P28	0.001	0.001	0.011	0.011	-0.012	-0.010	0.010	0.018	0.004	-0.001	0.000	-0.004	-0.008	**0.038**	-0.002	-0.005	0.002	-0.002	0.015	**0.023**	-0.001	**0.027**	-0.004	0.000	-0.001

Table S3: Analysis of molecular variance (AMOVA) to assess, at relevant epidemic periods (end/beginning of the epidemic), the effect of spore type giving rise to the isolate on the genetic structure of the pycnidiospore and ascospore subpopulations (see Fig. 1) collected in Thiverval-Grignon, France.

A - Each ascospore subpopulation collected from wheat debris at the beginning of an epidemic (P08, P28) was compared with the pycnidiospore subpopulation collected at the end of the previous cropping season from D+ plots (P04, P21, respectively), and with the pycnidiospore subpopulation collected from the D_0 plot (P07, P27, respectively). B - Each ascospore subpopulation collected from wheat debris at the beginning of an epidemic (P01, P08, P28) was compared with the pycnidiospore subpopulations collected during the same period from D+ plots (P02, P09, P29, respectively), and D_0 plots (P05, P13).

A	Df	Sum of squares	Variance components	% variation	*P*-value	F_{ST}
P08 (ascospore) vs. P04 (D+)						
Between subpopulations	1	2.899	0.031	1.20	0.296	0.012
Within subpopulations	28	71.034	2.537	98.80		
Total	*29*	*73.933*	*2.568*			
P08 (ascospore) vs. P07 (D_0)						
Between subpopulations	1	1.418	-0.034	-1.90	0.721	-0.019
Within subpopulations	33	60.782	1.841	101.90		
Total	*34*	*62.200*	*1.808*			
P28 (ascospore) vs. P21 (D+)						
Between populations	1	1.486	-0.005	-0.29	0.664	-0.003
Within populations	134	245.095	1.829	100.29		
Total	*135*	*246.581*	*1.824*			
P28 (ascospore) vs. P27 (D_0)						
Between subpopulations	1	2.732	0.052	3.35	**0.045**	0.033
Within subpopulations	65	98.283	1.512	96.65		
Total	*66*	*101.015*	*1.564*			

B	Df	Sum of squares	Variance components	% variation	*P*-value	F_{ST}
P01 (ascospore) vs. P02 (D+)						
Between subpopulations	1	1.9	-0.009	-0.40	0.638	-0.004
Within subpopulations	89	206.2	2.317	100.40		
Total	*90*	*208.1*	*2.308*			
P01 (ascospore) vs. P05 (D_0)						
Between subpopulations	1	2.0	0.010	0.57	0.279	0.006
Within subpopulations	69	121.2	1.756	99.43		
Total	*70*	*123.2*	*1.766*			
P08 (ascospore) vs. P09 (D+)						
Between subpopulations	1	2.2	0.054	3.35	0.166	0.033
Within subpopulations	30	46.8	1.560	96.65		
Total	*31*	*49.0*	*1.614*			
P08 (ascospore) vs. P13 (D_0)						
Between subpopulations	1	2.0	0.135	10.11	0.118	0.101
Within subpopulations	11	13.2	1.200	89.89		
Total	*12*	*15.2*	*1.335*			
P28 (ascospore) vs. P29 (D+)						
Between subpopulations	1	0.8	-0.004	-0.42	0.659	-0.004
Within subpopulations	117	129.0	1.103	100.42		
Total	*118*	*129.8*	*1.098*			