Long-term surveillance suggests multiple hybridization events by nuclear reassortment and accelerated intercontinental spread of wheat yellow rust.

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Summary

- Drivers of evolution of crop pathogens leading to novel diversity and long-distance dispersal (LLD) may have strong implications on food security and sustainable plant disease control at global scales. However, consolidated evidence is often lacking due to absence of regular pathogen surveys beyond national capacities.
- Our study documents world-wide connectivity between populations of *Puccinia striiformis*, causing yellow rust on cereals and grasses. Twelve cases of inter-continental spread of wheat yellow rust, including seven cases with major impact on

disease epidemics in recipient areas, were detected from genotypic analyses of 3240 pathogen samples from 41 countries at six continents.

- In three cases, the most plausible mechanism for novel pathogen diversity was somatic hybridization via nuclear reassortment between co-existing lineages on shared host cultivars, spreading by LDD from Europe to South America and Australia, respectively, a few years after first detection. Several high-impact incursions from South Asia into East Africa were also observed, including PstS16 with a dramatic impact on wheat breeding programs of global relevance.
- The present study stresses an urgent need for coordinated crop pathogens monitoring across borders. Only global efforts will enable prevention and control of pathogens that represent major challenges for food security at regional and global levels.

Key words

Puccinia striiformis, hybridization, world-wide spread, trajectory simulations, global surveillance, haplo-phased genome, SSR haplotype

Introduction

An increase in human travel and trade and the escalating speed of changing climate and weather patterns have renewed attention on disease epidemiology in humans, animals and crop plants, e.g., the US National Academies of Sciences (Olsen et al., 2011), the United Nations Food and Agriculture Organisation (FAO, 2024). This is exemplified by human vulnerability to pandemics as highlight by the coronavirus SARS-CoV-2 (Dillon et al., 2021; Chavda et al., 2022). Long distance dispersal (LDD) is an important epidemiological feature of many pathogens including those affecting crops, because despite LDD events potentially being rare they can be hugely impactful on crop production (Brown and Hovmøller, 2002; Carvajal-Yepes et al., 2019). Recent reviews suggest that LDD events of cereal rust pathogens are increasing in frequency at the intercontinental scale, possibly due to intensified crop production, climate change resulting in milder winters in temperate regions, and increased travel and commerce (Park, 2015; Jin et al., 2020; Hovmøller et al., 2023). The negative impact of more frequent LDD events may delay or prevent meeting the 70% increase in food and agricultural production by 2050 demanded by global population growth (van Dijk et al., 2021).

One major obstacle for the detection of LDD events in plant pathology is the lack of regular and extensive pathogen population surveys beyond national capacities (Jeger and Pautasso, 2008; Ristaino et al., 2021), including common sampling protocols, analytic tools and data sharing (Patpour et al., 2022). This makes it difficult to establish the exact geographical and evolutionary origins of a new pathogen variant as it spreads into recipient areas. Population genetic and genomic analyses are useful approaches to calculate distances and migration rates within and among pathogen populations (Zhan et al., 2003; Ali et al., 2014a; Bebber et al., 2014). At the same time, tracking of individual multi-locus genotypes (MLGs) represents a powerful strategy for the detection of LDD events especially in clonal populations, which tend to preserve haplotypes (Hovmøller et al., 2002).

The rust fungi (*Pucciniales*) are the most species rich and complex order of plant pathogens, consisting of almost 8000 species. They are characterized by a true biotrophic lifestyle and are individually adapted to their host plant at species and cultivar levels (Flor, 1971; Anikster, 1985; Aime et al., 2017). *Puccinia striiformis* f. sp. *tritici (Pst)*, causing yellow (or stripe) rust in wheat, is dikaryotic, heteroecious and macrocyclic requiring two taxonomically unrelated hosts to complete its life cycle (Jin et al., 2010). However, sexual reproduction on the alternate host, *Berberis spp.,* is observed rarely in nature, even in western China (Wang et al., 2016), despite multiple reports of signatures of sexual reproduction in populations in the near-Himalayan region including China, which are considered the center of diversity for *Pst* (Duan et al., 2010; Ali et al., 2014b; Thach et al., 2016; Huang et al., 2021). Somatic hybridization by fusion of hyphae, leading to nuclear reassortment which may preserve the entire haplotype, has so far only been reported under experimental conditions in *Pst* (Little and Manners, 1967; Little and Manners, 1969; Lei et al., 2017), unlike the wheat leaf rust pathogen *P. triticina* (Park and Wellings, 2012; Sperschneider et al., 2023) and the wheat stem rust pathogen *P. graminis* f. sp. *tritici* (Li et al., 2019).

The use of molecular markers in recent time has contributed significantly to the understanding of the global landscape of wheat rust fungi. In North America, yellow rust was first discovered in 1915, although the founder population is not known (Line, 2002), however, random samples from the 1970s, 1980s and 1990s suggested a common origin of the North American and the European PstS0 population (Markell and Milus, 2008; Ali et al., 2014a; Thach et al., 2016). The initial spread of *Pst* from Europe to Australia in 1978, also PstS0, is well documented (Wellings and McIntosh, 1990; Hovmøller et al., 2008; Ding et al., 2021). The worldwide spread of two warm temperature-adapted strains of *Pst* in 2000 and following years (termed PstS1 and PstS2, respectively), may collectively represent the largest and most rapid spread of

a crop pathogen up until that time (Hovmøller et al., 2008). Timewise, these large-scale dispersal events of *Pst* coincided with the emergence of *Pgt* race "Ug99" in East Africa that was virulent on more than 80% of wheat lines developed by The International Maize and Wheat Improvement Center (CIMMYT) (McIntosh and Pretorius, 2011). In response, the Borlaug Global Rust Initiative (BGRI) was formed and laid the foundation for more extensive and regular wheat rust disease and pathogen surveys. These included Africa and Asia, which were connected with national rust survey programs in Europe and elsewhere by the establishment of the Global Rust Reference Center in 2008 (Hovmøller, 2011). This allowed analyses of the spread and evolution of *Pst* and *Pgt* across borders and continents, including the return of *Pgt* into Europe and connectivity to epidemics elsewhere (Patpour et al., 2022), including Western Siberia 2015-2016 (Shamanin et al., 2016) and East Africa (Olivera et al., 2015). In addition, independent surveys have reported large scale epidemiological consequences of new incursions of *Pst* into South America in 2017 (Anibal Carmona et al., 2019; Riella et al., 2024) and Australia in 2017-2018 (Ding et al., 2021).

The present study investigated drivers of evolution of the *P. striiformis* population at the global scale based on genotypic analyses of 3180 samples collected at four continents (37 countries) between 2009 and 2023, complemented by genotype information of additional 60 samples representing long-term surveillance efforts in Europe, North America (Milus et al., 2015a) and Australia (Ding et al., 2021). LDD events were investigated by the potential sharing of genotypes in putative source and recipient areas, considering the time of first detection as well as accumulation of genetic diversity in the considered populations. Likely evolutionary origin of successful LDD migrants were explored in detail in specific cases, including hypothetical hybridization events by nuclear reassortment, which has not been demonstrated in *Pst* under non-experimental conditions. The plausibility of wind-dispersal along hypothesized transmission routes was explored by conducting trajectory simulations, and considering typical spore survival times and mean wind patterns. The prospects for prevention and mitigation efforts to reduce the increasing impact of crop pathogen spread at intercontinental scale are discussed.

Materials and Methods

Collection and recovery of yellow rust samples

Pathogen sampling reflected the coordinated efforts in wheat rust surveillance facilitated by research programs within the Borlaug Global Rust Initiative, European Union research and

network initiatives as well as national programs in Europe and Australia and recent studies in North America **(Fig. S1).**

A total of 3180 samples of yellow rust mainly from wheat (and occasionally other cereals and grasses) were collected in 37 countries on four continents between 2009 and 2023 (Table S1). The number of samples varied across years and locations, reflecting occurrence and sampling efforts of the yellow rust disease. Two hundred thirteen samples from the Middle East and West Asia were provided by The Regional Cereal Rust Research Center (RCRRC), ICARDA, Turkey. An additional pre-existing 60 isolates served as references of previously identified *Pst* groups, and *Pst* populations in USA (Milus et al. (2015b) and Australia (Ding et al. 2021).

Recovery of incoming samples and multiplication of urediniospores followed the procedure by Thach et al. (2025), after which they were harvested, dried, and stored in liquid nitrogen (-196°C) or freezer (-80°C) until further use. For analysis of diversity over time and space, samples were grouped into time periods I (2009-2016) and II (2017-2023) and seven geographical regions, in addition to reference samples from North America, Australia and Europe.

DNA extraction and SSR genotyping

From 2009 to 2012, DNA was extracted from dried urediniospores (approx. 20 mg/sample) according to Thach et al. (2016). Since 2013, extraction was based on dried leaf segments with single rust lesions taken from multiplication plants or directly from incoming samples according to Thach et al. (2025). Genotyping was done using 19 simple sequence repeat (SSR) co-dominant markers and allele sizes were manually scored, allowing up to two missing markers per sample (Thach et al., 2025). Samples with allele sizes and/or combinations suggesting more than a single genotype were excluded from the dataset. Detection of novel or exotic genotypes in a region/country was confirmed by additional independent DNA extractions from original sample material and genotyping. Two hundred thirteen samples from the Middle East (2018-2020) collected by Turkey-ICARDA Regional Cereal Rust Research Center were SSR genotyped by EUROFINS, France. Scoring of allele sizes and data alignment to existing format were carried out at GRRC. Assignment of multilocus genotype identity (MLG #) was done for samples without missing data using poppr package version 2.9.3 implemented in the R environment (Kamvar et al., 2014; Kamvar et al., 2015); for samples with missing data at a maximum of two marker loci, new MLGs were considered in case scored allele sizes revealed additional variability, whereas missing observations were not taken

into account. Duplicate MLGs derived from the same bulk sample were removed from the dataset.

Definition of genetic groups

Nineteen previously defined genetic groups within *Pst* (PstS0-PstS18), each consisting of closely related MLGs and associated races (Thach et al., 2025), were used to illustrate regional pathogen differences and changes between two time periods, 2009-2016 and 2017-2023, respectively. Historically, the definition and naming of a new group has been inspired by the potential epidemic impact across large areas Hovmøller et al. (2010), and a diversification from previous world-wide groups by a significant number of alleles (typically five SSR alleles or more) and/or group-specific diagnostic markers (Walter et al., 2016; Ali et al., 2017). An updated summary of named *Pst* groups is available at the GRRC website: https://agro.au.dk/forskning/internationale-platforme/wheatrust/yellow-rust-tools-maps-and-charts/definitions-of-races-and-genetic-groups.

Phylogenetic analysis

The dataset for phylogenetic analysis was compiled by including one representative sample from each MLG (clone corrected). An unrooted phylogenetic Neighbor Joining tree was constructed to visualize global diversity within *Pst*, including the positions of major genetic groups defined as described above. The calculations were based on calculated genetic distances using poppr, 1200 bootstraps and cutoff at 50 (Kamvar et al., 2014), where Bruvo's distance for stepwise mutation for microsatellites was calculated according to Bruvo et al. (2004).

Population diversity and structure

To investigate the power of discrimination of applied markers, a genotype accumulation curve was calculated in the poppr R package based on samples without missing data (n= 2588), and random resampling 10,000 times without replacement (Kamvar et al., 2014) (Fig. S2).

F_{st} values representing divergence between the geographic populations in two time periods (I: 2009-2016 and II: 2017-2023) were calculated based on the full dataset (3180 samples) and a clone-corrected dataset with a single MLG representative per population and time period (283 samples) according to (Peakall and Smouse, 2006; 2012).

200 **Tests for somatic hybridization by nuclear reassortment**

201 Nuclear-specific haplotypes of hypothetical parental and hybrid isolates were inferred based on
202 the position of SSR markers and allele sizes on a chromosomal map derived from haplotype-
203 phased genome data of a PstS0 isolate (Plant Breeding Institute accession number 415
204 collected in 1982) (Schwessinger et al., 2018; Tam et al., 2025). The position of SSR markers
205 were determined by blast analysis (-word_size 12 -evalue 1 -dust no -outfmt 6) of 20 primer
206 sequence pairs against the PstS0 genome (Table S2) and visualized by a phenogram according
207 to Wolfgang et al. (2013), including the lengths of individual SSR alleles (Fig. S3). The
208 analyses took advantage of multiple hypothetical hybridization events involving shared SSR
209 haplotypes, as well as time and geo-referenced sampling supporting both temporal and spatial
210 dimensions of evolution within the considered geographical populations.

211

212 **Whole genome confirmation of a somatic hybridization event**

213 The hypothesized hybridization resulting in PstS10 inferred by SSR and PstS0 genome data
214 were validated by two newly generated reference assemblies of isolates representing PstS7 and
215 PstS10, respectively. The fully phased nuclear-assigned reference genome for an Australian
216 sampled PstS10 isolate (Ding et al. 2021) was generated as described previously for PstS0
217 (Tam et al., 2025) with the exception of using PacBio HiFi and Oxford Nanopore Technology
218 (ONT) R10.4 based long-reads and Hi-C as input for Verkko (Rautiainen et al., 2023). The
219 haplotype-phased reference genome of PstS7 used ONT R10.4.1 simplex reads and Hi-C with
220 hifiasm --ont (Cheng et al., 2025). To expand upon the MLG results at the genome level, we
221 performed comparative alignments between the nuclear-phased haplotypes of a proposed
222 somatic hybrid, PstS10, and those of its two hypothetical parental isolates PstS0 (Tam *et al.*,
223 2025) and PstS7. Pairwise whole-genome alignments were conducted using NUCmer v3.1 as
224 part of the MUMmer package (Marçais et al., 2018) with default settings. Only alignment
225 blocks with >75% identity and longer than 500 bp were retained. SNP counts and average
226 identity were computed from 1-to-1 alignments using MUMmer's DNAdiff v1.3. Dot plots
227 were generated using a script adapted from paf2dotplot.r
228 (https://github.com/moold/paf2dotplot). The chromosomal locations and allele sizes for PstS7
229 and PstS10 were also visualized in Table S2 and Fig. S3, respectively, and calculated amplicon
230 lengths were compared with SSR genotyping results.

231

232 **Virulence phenotyping for validation of recent common origin**

233 Successfully recovered migrant isolates from putative source and recipient areas were
234 virulence phenotyped to provide an independent assay for relatedness. An updated protocol
235 was followed using a 0-9 disease scoring scale according to (McNeal et al., 1971), where
236 infection types (IT) from 0-6 were generally considered incompatible (avirulent) and IT 7-9
237 compatible (virulent) (Thach et al., 2025). The interpretation of virulence/avirulence for each
238 isolate was generally based on two (or more) independent wheat lines carrying the
239 corresponding *P. striiformis Yr* resistance gene.
240

241 **Plausibility of wind-dispersal**

242 Plausibility of wind-dispersal was analysed by reviewing data from previous studies on related
243 fungal pathogens (Brown and Hovmøller, 2002; Isard et al., 2005; Meyer et al., 2017a; Meyer
244 et al., 2017b; Prank et al., 2019; Radici et al., 2022; Brodsky et al., 2023; Miedaner and
245 Garbelotto, 2024), analyzing long-term mean wind patterns, i.e., wind direction and speed for
246 times of overlapping wheat seasons at source and recipient areas based on the International
247 Research Institute for Climate and Society
248 (https://iridl.ldeo.columbia.edu/maproom/Global/Climatologies/Vector_Winds.html),
249 estimating typical spore survival times (Maddison and Manners, 1972; Rapilly, 1979; Aylor,
250 1986), and conducting a set of trajectory simulations using HYSPLIT (Stein et al., 2015) with
251 two different global-scale gridded meteorological datasets [NOAA's GDAS, 3-hourly temporal
252 resolution and 1 degree horizontal spatial resolution; ECMWF's ERA5, hourly temporal
253 resolution and 0.25 degree spatioal resolution (Hersbach et al., 2020) (see Fig. S4 for details).
254 Time-backwards trajectory simulations based on GDAS were conducted from locations in all
255 recipient areas to investigate origins of air masses that could have transported spores to recipient
256 areas where they were first detected. Time-forward simulations based on ERA5 were conducted
257 to analyze the plausibility of stepwise airborne transmission along two selected pathways
258 (Europe to South America via Northern Africa; and Central Asia to East Africa via the Middle
259 East).
260

261 **Results**

262 **Genotypic *Pst* diversity at global scales**

263 A total of 3483 samples collected from 41 countries on six continents were genotyped using
264 previously published SSR markers (Thach et al., 2025), which were all polymorphic except for
265 NRJN12. Of these samples, 136 were duplicates of identical genotypes/races that had been sub-
266 cultured from original bulk samples, and an additional 153 contained two or more genotypes per

sample. The 289 duplicate and mixed samples were excluded, leaving a total of 3180 for further analysis. An additional 60 reference samples representing comprehensive long-term national rust surveys in Australia, North America and Europe were included to illustrate genetic diversity at global scale (Fig. 1). A majority of the samples (91.7%) grouped into previously defined genetic groups (PstS0-PstS18). The remaining samples consisted of 74 highly diverse MLGs of distinct geographical origins, including areas in South Asia, where high diversity has been reported previously. Samples from non-wheat hosts in northern Europe, e.g., barley, wild grasses, and Triticale, formed a distinct and diverse branch in the tree, suggesting a different evolutionary origin. The 20 reference samples from North America contained six MLGs grouping within PstS0, PstS1_2, PstS18, and two unnamed groups. The 31 reference samples from Australia were contained in seven MLGs that grouped within PstS0, PstS1_2, PstS10 and PstS13, and the six reference samples from Europe and one from East Africa (1960-2006) represented PstS1_2, PstS3 and PstS4.

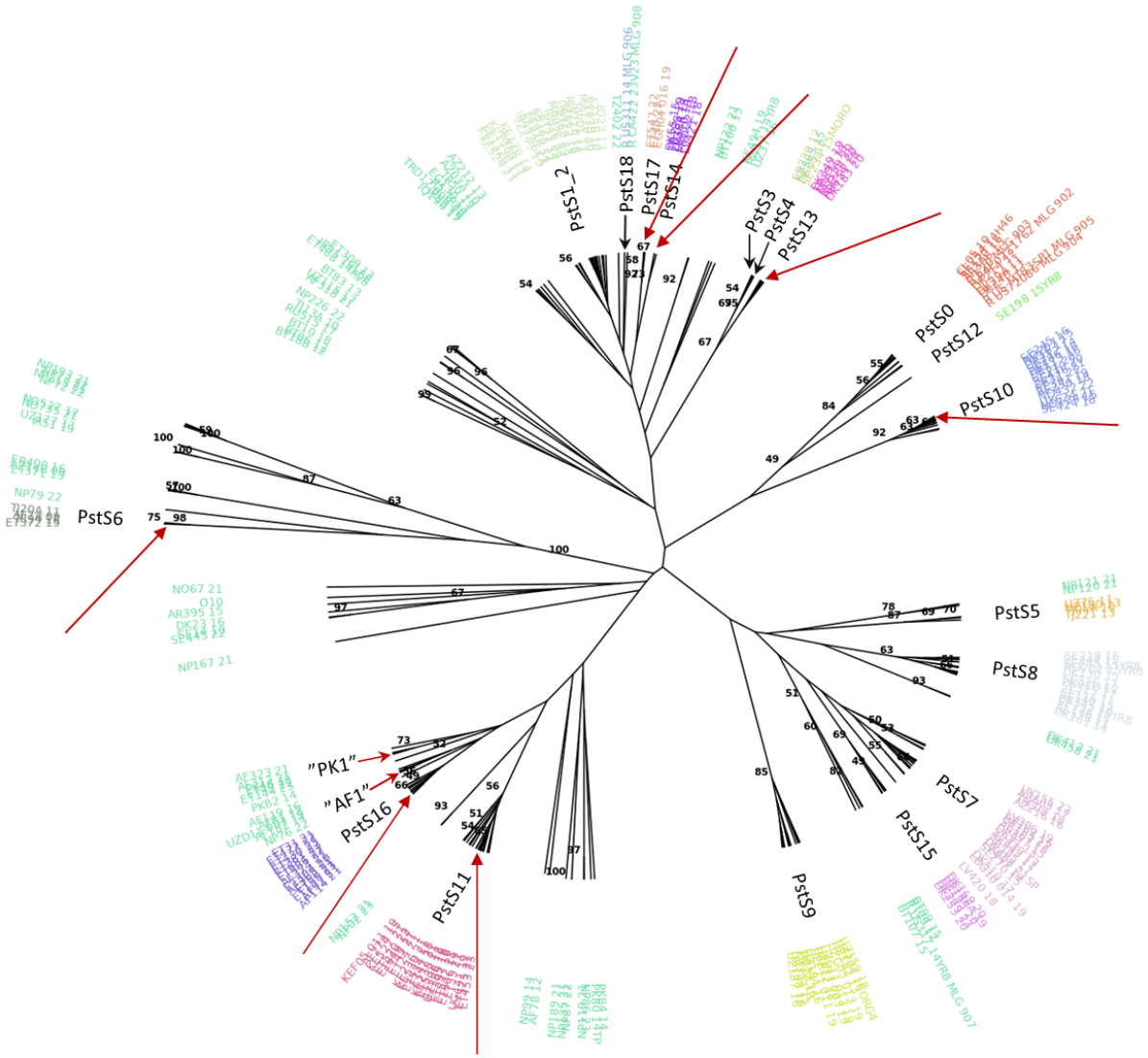

Fig. 1. Unrooted Neighbor Joining tree (clone-corrected) using Bruvo's distance showing the genetic relationship of 244 multi-locus genotypes (MLGs) representing world-wide sampling of *P. striiformis* in 41 countries on six continents (2009-2023), including reference isolates representing populations in Australia (1979-2021) and North America (1972-2023). Position of named genetic groups (PstS0-PstS18) and two temporary named groups (AF1, PK1) are indicated by different colours, typically consisting of clusters of multiple, closely related MLGs. Additional 74 unnamed, diverse MLGs were shown in greenish blue. Intercontinental migrants indicated by red arrows (within group position indicative).

The within-group diversity largely reflected time since first detection of the group, evolutionary origin, and likely prevalence of the disease and sampling intensity (Table 1; Fig. S2). The highest number of MLGs were observed in PstS1_2, first detected in East Africa in the 1970s, but relatively high numbers were also observed for groups emerging from populations with high

295 genetic diversity in Asia, e.g., PstS7, PstS8, and PstS11, despite low diversity at time of incursion
296 into Europe (PstS7, PstS8 in 2011) and East Africa (Psts11 in 2016) (data not shown).
297 Table 1. Diversity within world-wide genetic groups and geographic *Pst* populations defined
298 according to Table S1. Shaded values represent population, where each genetic group was first
299 detected.

	Overall diversity				Number of MLGs per population[1] (*n* =population size)						
Genetic group	Number of samples (n)	Number of MLG/ genetic	Shannon-Weiner index (H)	Frequency of prevalent MLG/ group	NEU (n=1086)	MEA (n=348)	EAF (n=914)	NWA (n=159)	SAS (n=283)	CWA (n=217)	SAM (n=173)
PstS0	57	7	1.95	54,4	5						4
PstS1_2	420	24	3.18	47,9		6	18	4	1	3	
PstS3	1	1	-	-						1	
PstS4	50	3	1.10	72,0	3	1					
PstS5	14	4	1.39	35,7						5	
PstS6	46	4	1.39	91,3			2		2	2	
PstS7	105	19	2.94	41,0	11	3		2		3	1
PstS8	128	15	2.71	51,6	15						
PstS9	139	14	2.64	53,2			1		1	14	
PstS10	600	16	2.77	82,2	15			1			1
PstS11	454	22	3.09	48,5		1	21		2	1	
PstS12	6	1	0.00	100,0	1						
PstS13	279	6	1.79	84,2	5			1			1
PstS14	239	6	1.79	72,0	5[2]	1		2[2]			1
PstS15	13	5	1.61	30,8	5						
PstS16	278	13	2.56	70,1			9		7		
PstS17	87	3	1.10	77,0	2	2	2				
Others	264	74	4.30		10	9	13		58	15	1
Total - named groups	2916	163			62	14	53	8	13	29	8
Grand total	3180	237	5.47		**72**	**23**	**66**	**8**	**71**	**44**	**9**

[1] Populations: NEU: Northern Europe, MEA: Middle East, EAF: East Africa, NWA: Northwest Africa, SAS: South Asia, CWA: Central-West Asia, SAM: South America.
300 [2] PstS14 first detected in NEU in 2015 (one single sample), where no surveys were conducted in NWA. PstS14 was highly dominant NWA from 2016 and onwards.

301

302 The distributions of isolates within the named genetic groups (PstS0-PstS17) were highly
303 divergent in the two time periods considered (2009-2016 and 2017-2023, respectively), and
304 across the considered geographical areas (Northern Europe, Middle East, East Africa,
305 Northwest Africa, South Asia, Central-West Asia, South America), which suggested dynamic
306 pathogen changes during time and significant geographic subdivisions since 2009 (Fig. 2).
307 Note that reference samples from North America and Australia, which represent historic key
308 isolates from these continents rather than systematic sampling in the two periods, were not
309 included in these analyses.

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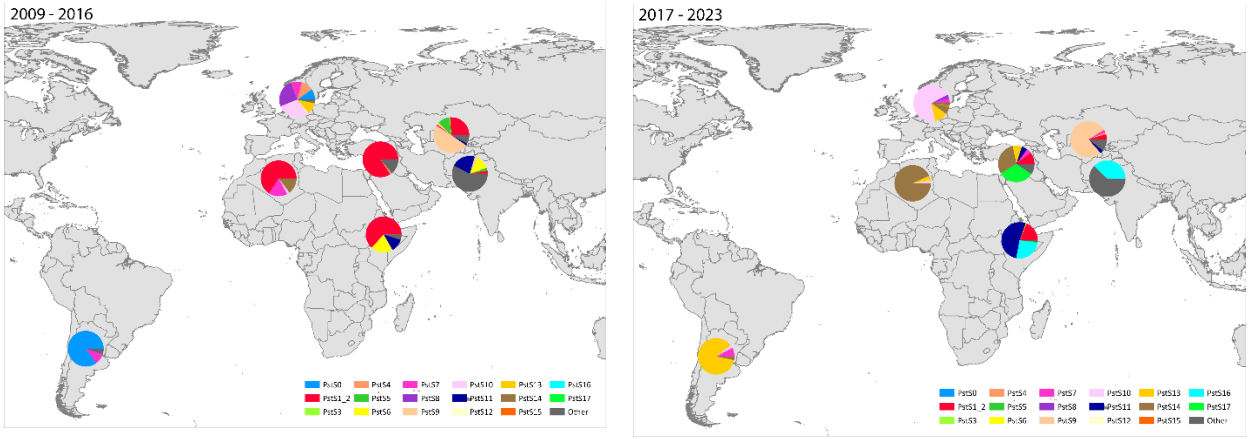


Fig. 2. Frequency of *P. striiformis* genetic groups in geographically distinct populations in period I (2009-2016, n= 921) and period II (2017-2023, n= 2259).

The distinct geographical distribution of genetic groups was striking. The aggressive and warm temperature-adapted group (PstS1/PstS2) was prevalent across Africa and the Middle East in period I (2009-2016), but absent from South America, Europe and South Asia. PstS5 and PstS9 were only detected in Central Asia. PstS0, the prevalent genetic group in Europe up to 2010, was only detected in Europe, South- and North America and Australia. PstS8 was prevalent in northern Europe until 2016 and was not detected in other areas. PstS10 has been the most prevalent group in Europe since 2014 but has not been detected in East Africa or South- and Central Asia. These overall differences were reflected by corresponding F_{st} values based on an analysis where MLGs were grouped according to geographic population and sampling period (Table S2). Additional analyses based on clone-corrected data confirmed these trends but pointed towards additional connectivity between Central-West Asian populations and African and South Asian populations, respectively.

The limited differentiation between Middle East and East Africa/ NW Africa, and between South Asia and East Africa suggests sufficient migration to maintain low differentiation between these populations. The replacement of the prevalent PstS0 population in South America by a population dominated by PstS13 since 2017 represents the most dramatic temporal population shift between periods I and II (Fst= 0.341).

The dataset also revealed several cases of emergence of novel, distinct genetic groups. For example, PstS10 containing a single MLG emerged in Europe in 2012 and became widespread accounting for 82% of PstS10 samples (Table 1). This was followed by the emergence of 14 additional, closely related MLGs in the European population. The emergence of PstS13 and

PstS14 in 2015-2016 in the European population represents two additional, distinct genetic groups that were not readily explained by mutation or migration events.

Identification of hypothetical somatic hybrids

Our genotypic data for PstS10, PstS13 and PstS14 were consistent with a hypothesis of somatic hybridization by nuclear reassortment of isolates of pre-existing lineages within the European population. First, allele sizes detected within samples representing MLGs of the first detected cases of PstS10, PstS13 and PstS14 were compared with samples representing all named genetic groups detected within Europe. This revealed that most MLGs and genetic groups were excluded as potential parent(s) due to mismatching allele sizes (Table S3). The next step involved inference of haplotypes of hypothetical parental pairs and hybrid MLGs by alignment with the PstS0 haplotype-phased reference genome, and assignment of the position of SSR markers on a genetic map, which revealed the genome derived SSR haplotype of PstS0 (Table S2, Fig. S3) as well as the haplotype based on observed SSR allele sizes (Table S6). Nucleus haplotype A of prevalent European races in PstS0 provided a 100% match with a nucleus haplotype of PstS10, where the second haplotype (B) provided a 100% match with a nucleus haplotype represented by the most prevalent MLG in PstS7. These results were confirmed by independent genomic data of isolates representing the parental groups of PstS0 and PstS7, and the hybridization resulting in PstS10 (Fig. S5). Similarly, we obtained 100% nucleus haplotype matches for PstS13 and PstS14, revealing that haplotype B in PstS7 was involved in the three hybridization events, however, arising from two different MLGs within PstS7. A schematic representation of the sequence and network of hybridization events is presented in Fig. 3, including dotplots of the pairwise genome alignments and total SNP counts and average identity among the nuclear phased haplotypes of the PstS10 hybrid and the likely parental isolates.

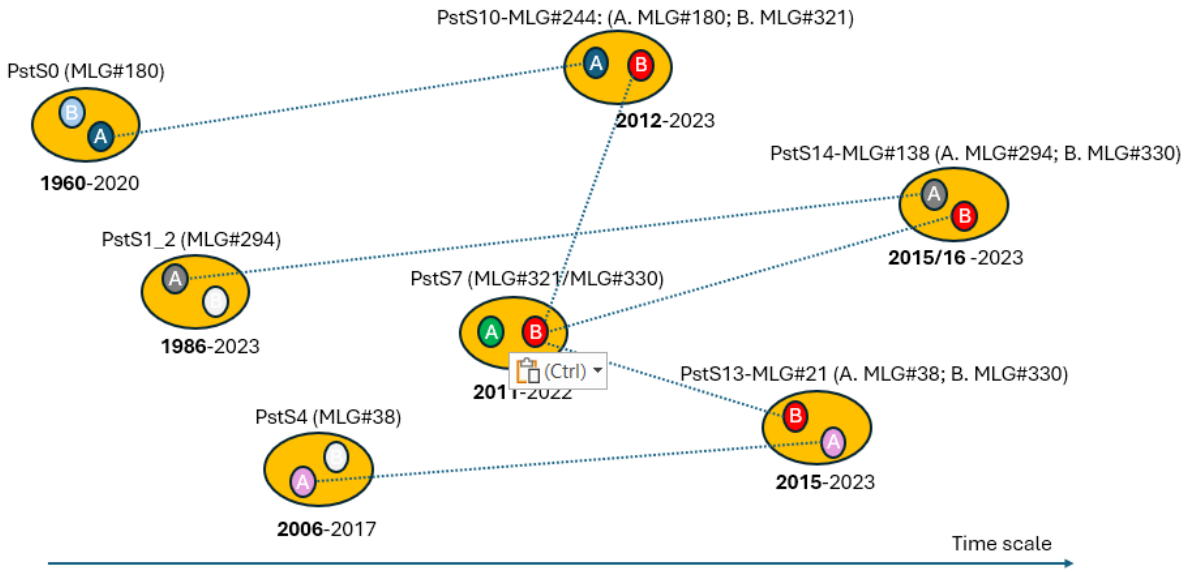


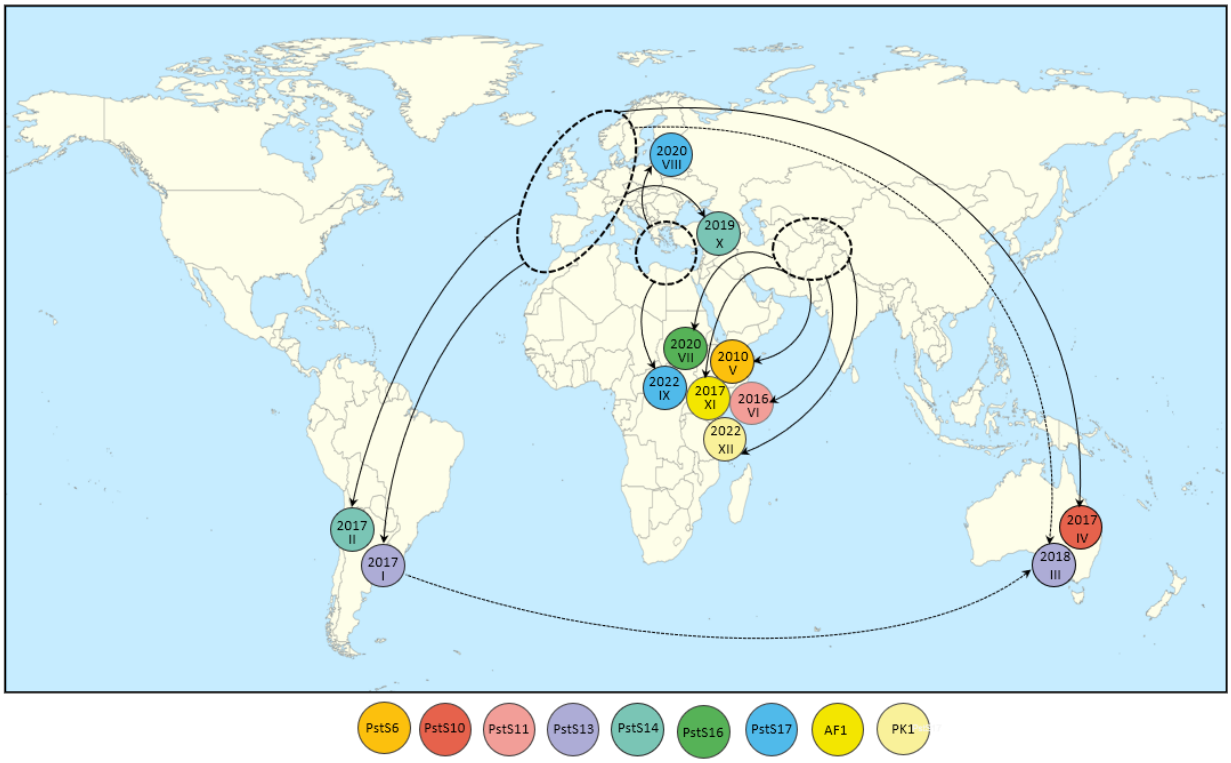
Fig. 3. Schematic representation of three independent hybridization events supported by observed SSR multilocus genotypes, pairwise genome alignments, and derived nuclear haplotypes. PstS10 was first detected in northern Europe (2012) in an area where the respective MLGs of PstS0 (#180) and PstS7 (#321) were prevalent on shared hosts of bread wheat. PstS13 was first detected in 2015, where a single MLG (#21) was detected at multiple locations in Europe, often on Triticale and rarely on winter wheat. The relevant MLG of PstS4 (#38) was detected almost exclusively on Triticale, while the MLG of PstS7 (#330) was detected widespread on triticale as well as bread wheat. PstS14 (MLG#138) was first detected in Europe in 2015/ NW Africa 2016 (not surveyed in 2015), the involved MLGs of PstS7 (MLG#330) and PstS1_2 (MLG#294) being prevalent in southern Europe/NW Africa at that time. First and last sampling years for individual genetic groups in the dataset are indicated (years before 2009 represented by historic reference samples).

Long distance dispersal events

The hypothesis of migration events at intercontinental scale was investigated by analyses of first detection of specific genetic groups and distinct MLGs within these in the seven geographical populations (Fig. 4). In three cases, first detection was associated with novel evolutionary origin by hybridization of pre-existing MLGs of divergent genetic groups within the considered geographical population, i.e., PstS10 (PstS0 and PstS7), PstS13 (PstS4 and PstS7), and PstS14 (PstS1_2 and PstS7). These were first detected in Europe and NW Africa between 2012 and 2016 (Fig. 4).

We documented 12 cases of exotic incursions of *Pst* at intercontinental scales (I-XII), including seven cases where exotic incursions had major impact on disease epidemics in

387 recipient areas. Migration direction and events were hypothesised based on year of first
388 detection / hybridization event in source and recipient areas, as well as prevalence and
389 diversity within considered genetic group (Table 1, Table 2, Figure 4).
390



391
392 **Figure 4.** Documented inter-continental incursions of *P. striiformis* 2009-2023, based on
393 sequential resampling of multilocus SSR genotypes/pathogen races on different continents.
394 Dotted circles represent putative source areas, where considered *Pst* group and MLGs defined
395 by 18 polymorphic SSR markers were first detected, arrows represent putative transmission
396 routes, colored circles represent first year of detection on the recipient continent and the
397 corresponding ID of individual MLGs. Details of individual dispersal events and individual
398 migrant MLGs are presented in Table 2. Dotted arrows: PstS13-MLG #21 present at two
399 continents at time of incursion into Australia. The positions of circles and arrows on the map
400 are indicative.
401
402 Table 2. Inter-continental *P. striiformis* dispersal events (I-XII) within a 15-year period (2009-
403 2023) documented by source and recipient areas of unique multi-locus genotypes (MLGs),
404 genetic groups and race phenotype.

Dispersal event (cf. Fig. 4)	Migrant (MLG #)	SSR genetic group	First detected (population and year)	Reference	Recipient population and year	Race confirmation in source and recipient population)[3]
I	21	PstS13	Europe (2015)	Ali et al. 2017	South America (2017)[2]	**Confirmed** : -,2,-,-,-,6,7,8,9,-,-,-,-,-,-,-,-,-,AvS,-
II	134	PstS14	Europe (2015)	Hovmøller et al. 2017 [1]	South America (2017)[2]	-
III	21	PstS13	Europe (2015)	Ali et al. 2017	Australia (2018)[2]	-
IV	244	PstS10	Europe (2012)	Ali et al. 2017	Australia (2017)[2]	-
V	8	PstS6	Central Asia (2009)	Ali et al. 2017	East Africa (2010)[2]	**Confirmed**: 1,2,-,-,-,6,7,-,9,-,-,17,-,-,27,-,-,AvS,-
VI	78	PstS11	Central Asia (2012)	Hovmøller et al. 2018 [1]	East Africa (2016)[2]	**Confirmed**: -,2,-,(4),-,6,7,8,-,-,-,17,-,-,27,32,-,AvS,-
VII	205	PstS16	South Asia (2017)	Hovmøller et al. 2023 [1]	East Africa (2020)[2]	**Confirmed**: 1,2,3,(4),-,6,7,8,9,-,-,17,-,25,27,32,-,AvS,-
VIII	170	PstS17	Middle East (2018)	Hovmøller et al. 2019 [1]	Europe (2020)	**Confirmed:** -,2,-,-,-,6,7,8,-,-,-,17,-,-,-,32,(Sp),AvS,Amb
IX	170	PstS17	Middle East (2018)	this paper	East Africa (2022)	**Confirmed:** -,2,-,-,-,6,7,8,-,-,-,17,-,-,-,32,(Sp),AvS,Amb
X	138	PstS14	Northwest Africa (2016)/ Europe (2016)	this paper	Middle East (2019)	-
XI	103	AF1	Central Asia (2012)	this paper	East Africa (2017)	**Confirmed**: 1,2,-,-,[-],6,7,8,9,-,-,17,[-],-,27,-,-,AvS,-
XII	185	PK1	South Asia (2017)	this paper	East Africa (2022)	-

[1] Annual reports from Global Rust Reference Center (accessible: www.wheatrust.org)

[2] High impact event in recipient areas

[3] Figures and symbols designate virulence and avirulence (-) corresponding to Pst resistance genes: *Yr1, Yr2, Yr3, Yr4, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr24, Yr25, Yr27, Yr32* , and the resistance specificity of Spalding Prolific (Sp), Avocet S (AvS) and Ambition (Amb).

In all cases, we were able to detect likely geographical source areas, including two specific MLGs spreading from Europe into South America in 2017 (I and II, Table 2), i.e., PstS13 (MLG #21) and PstS14 (MLG #134), MLG #21 spreading into Australia (III, Table 2) the following year (2018). PstS10 (MLG #244), the most common genotype in Europe since 2016, was also detected as an exotic incursion in Australia in 2017 (IV, Table 2). MLGs within three additional and highly divergent genetic groups, PstS6 (MLG #8), PstS11 (MLG #78) and PstS16 (MLG #205), detected in Central/South Asia in 2009, 2012 and 2017, were observed for the first time in East Africa (Ethiopia) in 2010, 2015 and 2020, respectively (V, VI, VII, Table 2). PstS17 (MLG #170), first detected in the Middle East in 2018 and since then prevalent in this region, was first detected at low frequency in northern Europe (Latvia, 2020, VIII, Table 2) and in East Africa (Ethiopia, 2022, IX, Table 2). PstS14 (MLG138), first detected in Europe and NW Africa in 2015/2016, was detected in the Middle East in 2019 (X, Table 2), later becoming dominant in local areas in West Asia, e.g., Iraq, where a single MLG in PstS14 made up 100% of samples from multiple locations and host cultivars (2021, 2022). Two MLGs, which have not yet been assigned a named genetic group, detected in Afghanistan, 2012 (temporary designation AF1, MLG #103) and Pakistan, 2017 (temporary designation PK1, MLG #185), respectively, were observed in East Africa in 2017 (Kenya) and 2022 (Ethiopia), respectively (XI, XII, Table 2). These genetic groups, including PstS17, have remained at low frequency to date in recipient areas in Northern Europe and East Africa.

We grouped the dispersal events according to similarities in source and recipient areas, resulting in seven potential intercontinental atmospheric transport routes (see Table S5). Results from HYSPLIT trajectory simulations, analyses of mean wind patterns in combination with wheat growth timing and spore survival times, as well as previous evidence from related fungal

pathogens lead us to hypothesize that (i) wind dispersal was not plausible for the two dispersal events, i.e., "Europe to Australia" (route 3) and "South America to Australia" (route 2), where accidental human-mediated transmission seems more likely; (ii) wind dispersal was highly unlikely for direct movement from Europe to South America (route 1) and from Central/South Asia directly to East Africa (route 4), although the data does not exclude the possibility of indirect, stepwise airborne transmission (via northern Africa and the Middle East/Arabian Peninsula, respectively); (iii) wind dispersal was plausible for the three dispersal routes, Middle East to Europe (route 5), Middle East to East Africa (route 6) and Europe/North Africa to the Middle East (route 7).

Discussion

The potential impact of rare events of long-distance dispersal (LDD) of plant pathogens on plant health has been known for decades, e.g., Brown and Hovmøller (2002), Smart and Fry (2001), Ristaino et al. (2021), Jones (2021). Despite this, in the vast majority of cases the exact source and recipient areas are not known. The focus has often been on high impact examples, e.g., overview for *Pst* on wheat by Jin et al. (2020) rather than cases with insignificant or no impact which may be overlooked and thereby contribute to underestimation of the frequency of LDD of crop pathogens.

The present study was based on unique and systematic wheat rust surveillance efforts over 15 years facilitated by the formation of the Borlaug Global Rust Initiative (BGRI) and the Global Rust Reference Center in 2008. This network approach has taken advantage of the engagement of multiple collaborators across the world in coordinated wheat rust sampling (Table S7), the use of standardized pathogen phenotyping and genotyping methodologies (Thach et al., 2025), as well as application of commonly agreed data management and analytic tools (Hovmøller, 2011). Altogether, the sampling areas covered major wheat growing areas in 37 countries on seven large geographical areas in Africa, Asia, Europe, and South America, and was further connected with North America and Australia through reference samples collected in major long-term national rust surveillance initiatives. Our study thereby represents the largest and most comprehensive survey in time and space of a major crop pathogen (Savary et al., 2019).

We confirmed at least 12 intercontinental LDD dispersal events on wheat, where we had distinct multi-locus genotype data, and in many cases also phenotypic virulence data, suggesting a recent common evolutionary origin for samples in both source and recipient areas. In seven cases, the dispersal events resulted in severe and widespread disease epidemics in recipient areas (Anibal Carmona et al. (2019) Riella et al. (2024); Ding et al. (2021), whereas

five cases resulted in only minor impact on rust epidemics in recipient areas (i.e., rare detection of the corresponding genetic groups in following years). In three cases, PstS10, PstS13, and PstS14, the first detected and most common MLGs representing each of these groups was hypothesized to originate via asexually by somatic hybridization and nuclear reassortment. This provided a unique opportunity to identify the evolutionary mechanism and source of migrants, in addition to identifying the geographical area of first detection. Our conclusions concerning the hypotheses of LLD dispersal in the latter three cases, versus an alternative hypothesis of hybridization of "local" strains in recipient areas, were in all cases supported by lack of detection of matching parental isolates of the hypothesized hybrids in recipient areas (Australia and South America), despite extensive and widespread survey activities (www.wheatrust.org; Anibal Carmona et al., 2019; Ding et al., 2021; Riella et al., 2024). Our data suggest additional LDD events, for which we were unable to track source areas for the observed MLGs, e.g., the emergence of MLG #321 (PstS7) in Argentina in 2018 following first detection in Europe (2011) and Northwest Africa (2013-2014), MLG #247 (PstS10) in Uruguay in 2021, and MLG #175 (PstS17) in northern Europe and East Africa. However, these observations most likely followed the dispersal paths of other MLGs of documented source origin, e.g., PstS7-MLG #321 and PstS10-MLG #247 (route 1), and PstS17-MLG#175 (route 5), but final conclusions would require additional samples from potential source areas that were not represented in the dataset. The multiple cases of LDD between continents, following previous reports of high-impact incursions, e.g., PstS7 (Warrior) and PstS8 (Kranich) from the near-Himalayan region into Europe in 2011 (Hovmøller et al., 2016), and PstS1 into North America and Australia, and the incursions of PstS2 into the Middle east, parts of Asia and Europe between 2000 and 2005, stresses a trend towards accelerated and impactful LDD spread of the yellow rust disease world-wide.

Our findings represent a new aspect of yellow rust epidemiology. Unlike other cereal rust fungi, somatic hybridization in *Pst*, leading to nuclear reassortment, and/or parasexual exchange of genetic material has only been reported under experimental conditions. Already in the 1960s, mixtures of isolates of urediniospores of different races suggested that somatic hybridization by fusion of hyphae and subsequent nuclear reassortment was the likely source of new race variants. More recently, cross infection studies on wheat and barley using paired experimental isolates also concluded that somatic recombination may take place, however, these results could only be explained by assuming chromosome reassortment and crossovers within/among parental isolates (Lei et al., 2017). Earlier, Park and Wellings (2012) concluded that somatic hybridization was likely for many rust species and *formae speciales*, the process

being observed in nature within and between rust species on Linum, poplar, Senecio, several grass species, and leaf rust on wheat, although the exact mechanisms were generally not well understood. Our data suggest that somatic hybridization may be a more significant source of agriculturally-relevant genetic diversity than sexual recombination for yellow rust, and help explain why the pathogen is rarely detected on the alternate host *Berberis vulgaris* (Jin et al., 2010; Wang et al., 2016), in the near Himalayan region or elsewhere, despite high genetic diversity (Duan et al., 2010; Ali et al., 2014a; Thach et al., 2016; Huang et al., 2021).

In the present study, putative hybridization events in *Pst* were identified by positioning SSR markers and allele sizes on a chromosomal map based on a haplotype-phased genome of an isolate in the genetic group PstS0 (Tam et al., 2025). This, combined with careful analysis of MLGs generated with consistently applied methodologies (Thach et al., 2025), revealed three major hybridization events giving rise to PstS10, PstS13 and PstS14. Importantly, we confirmed the PstS10 hybridization event by comparing the haplotype-phased nuclear assigned genomes of the potential parents. In more detail, the prevalence of matching MLG haplotypes of PstS0 and PstS7 suggested a hybridization event resulting in PstS10 somewhere in northern/western Europe on bread wheat. In contrast, a rare MLG in PstS7 (#330, <10%) that was sampled from both bread wheat and Triticale, and a frequent MLG in PstS4 (#38, > 95%) that was almost exclusively sampled from Triticale, were uniquely detected as parental haplotypes forming PstS13, suggesting that hybridization most likely took place on Triticale. The resulting MLG (# 21) of PstS13 was frequently detected on Triticale and durum wheat, as well as spring wheat, but rarely on winter wheat. One of the putative parents of PstS14, PstS1_2 MLG #294, was detected only in NW Africa, coinciding with the presence of a matching second parent (PstS7 MLG #330) in the same area in 2014. Interestingly, PstS14 contributed 100% of samples in surveys in three consecutive years in NW Africa since 2016, whereas this group was observed only sporadically elsewhere, i.e., 2016 (six samples), 2017 (38 samples) and 2018 (54 samples), providing additional support that PstS14 indeed emerged by hybridization in this area. In fact, our data revealed two additional cases of 100% matches between hypothesized parental pairs and hybrids, which remained at low prevalence in the European *Pst* population, and one case of hybridization in the North American population (data not shown).

In four high-impact and three low-impact cases, the conclusion of very recent dispersal events was supported by identical virulence phenotypes in source and recipient areas based on assays comprising wheat experimental lines representing more than 25 different host resistance specificities (Thach et al., 2025). Because the evolution of virulence in plant pathogenic fungi,

including *Pst* may be significantly higher than average mutation rates (Hovmøller and Justesen, 2007), the existence of an exact match in virulence phenotype is an additional indication of direct connection between such populations in an evolutionary time perspective. Based on the 12 individual dispersal events, we hypothesized seven putative transmission routes, for which the plausibility of wind dispersal was investigated through HYSPLIT trajectory simulations based on finely resolved global meteorological data, testing different spore survival times in the atmosphere, and also through analyses of wheat growing timing, monthly mean winds during those periods where the empirical data suggested that LDD could have taken place, and recent evidence from related pathogens. Our analysis points to both human mediated and windborne dispersal playing important roles in intercontinental dispersal of wheat yellow rust. Results indicate that airborne dispersal is plausible between the Middle East and East Africa as well as Europe. This is in line with the high genetic similarity of *Pst* populations in the Middle East and East Africa (Table S2, F_{ST}) that also indicates frequent gene flow between these regions and could explain several reported incursion events (Fig. 2 & Table 2). The recent detections of strains Pst6, Pst11, Pst16, Pst17, AF1 and PK1 in East Africa after previous detections in the Middle East and Central/South Asia, as reported here, provide supportive evidence for a previously hypothesized Rift Valley Incursion pathway for rust pathogens (Meyer et al., 2017a). Direct dispersal from Central/South Asia to East Africa seems highly unlikely, however the possibility of stepwise airborne dispersal via the Middle East cannot be outruled. Since PstS6 and PstS16 were not detected in the Middle East, and PstS11 was not detected in this region until 2019, three years after first detection in East Africa, the stepwise model may be less likely, although relatively low sampling intensity in critical areas on the Arabian Peninsula and the Horn of Africa should be taken into account, as well as the stochastic nature of these long-range LDD events.

Direct airborne dispersal from Europe to South America or from Central/South Asia to East Africa was highly unlikely, and a more comprehensive modelling study is required to test the hypothesis of stepwise airborne transmission. For the two dispersal events Europe to Australia and South America to Australia, wind dispersal was not plausible, indicating human mediated transport and emphasizing the importance of quantifying risks due to unintended human transmission of exotic pathogenic organisms via trade and travel. The investigation of windborne dispersal summarized here aimed at developing initial hypotheses about which dispersal mechanisms might be plausible for each incursion event. Testing as part of future work would require a large-scale modelling study involving detailed dispersion simulations that include environmental and epidemiological dynamics at source and target regions, along with systematic

screening of sensitivities to spore survival times, release locations, and release times. More detailed experimental data on spore survival times during atmospheric transport obtained from large samples of viable spores, to estimate more reliably the fraction of spores, out of extremely large numbers released during outbreaks, and a continued extension of global surveillance networks are essential for further improving our understanding and capacity to predict and mitigate risks from long-range exotic incursion events.

The connectivity between populations of airborne pathogens is probably especially evident in global cropping systems like wheat, grown on six continents. This gives rise to several scientific questions as well as sustained need for coordinated pathogen surveillance and early-warning systems at both regional and global levels. Access to reference laboratories and testing facilities is crucial. These facilities can assess the vulnerability of crop breeding material and deployed cultivars against new and aggressive pathogen strains before they become widespread. We show here that somatic (asexual) hybridization both takes place and seems to be common in populations of *Pst* in nature despite not being previously reported outside of experimental facilities. The fact that such hybrids quickly replaced pre-existing pathogen populations in Europe, Australia and South America suggests that new epidemiological insights into the actual mechanisms of hybridization at both cellular and genetic levels are required. Moreover, it suggests that such hybrids can be potentially superior in fitness to their parents, which may relate to changed interactions with various components and mechanisms of host resistance.

Our data suggest that the risks and speed of pathogen evolution driven by mutation and hybridization may correlate positively with the size and areas affected by ongoing epidemics. In turn, this may influence the probabilities of further hybridization and risks of unintentional pathogen spread at local, regional and global levels. Thus, there is an imminent and urgent need to sustain research and development within the area of plant breeding and disease epidemiology (experimental as well as modelling efforts) to prevent future epidemics so far as possible. In parallel, it is important to raise awareness of the risks of unintentional spread of important crop pathogens, e.g., by travellers, to enhance food security in agroecological settings with rapidly changing climate and weather patterns and increasing political instability.

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Competing interests

None declared

Author contributions

DH and MSH conceived and designed the surveillance program; TT carried out the experimental SSR genotyping work under the supervision of AFJ, including data alignment and quality check and population genetic analyses; VR assisted in genotyping of samples from South America; JRA carried out virulence phenotyping of hypothetical migrant isolates a supplied HQ DNA of PstS7 for sequencing; JGH developed the web-based data management and display system for world-wide prevalence of *Pst* genetic groups; KN supplied raw SSR data for Middle East samples 2018-2020; RP was curator of reference samples representing the Australian *Pst* population 1979-2021, undertook race analysis, and was responsible for associated funding; RT and MM performed the sequencing and genomic data analyses of

PstS10 hybrid and parental isolates; BS and JR contributed to genomic data analysis, supervised and secured funding for the genomic work and provided extensive feedback on the manuscript; PS RT and MM performed the sequencing and genomic data analyses of PstS10 hybrid and parental isolates; BS and JR supervised and secured funding for the genomic work; PS supervised the PhD work of VR in South America; MME conducted trajectory simulations of the plausibility of wind dispersal of spores based on meteorological datasets; MSH was curator of samples hosted by the GRRC, supervised project activities anchored at GRRC, wrote the first draft of the manuscript; all authors revised and accepted the final manuscript.

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Data availability

All raw sequencing data generated in this study have been submitted to NCBI BioProject database under accession number PRJNA1256629. The SSR genotypic data of the 3180 isolates included in the present study are available at https://doi.org/ (pending)

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