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Population structure and temporal maintenance of *Puccinia striiformis* in the Himalayan region of Pakistan

Sajid S. Ali, Pierre Gladieux, Hidayatur Rahman, Shahab Saqib, Muhammad Fiaz, Habib Ahmed, Marc Leconte, Angelique Gautier, Mogens Stovring Hovmoller, Jerome Enjalbert, et al.

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Disease Risk and Food Security

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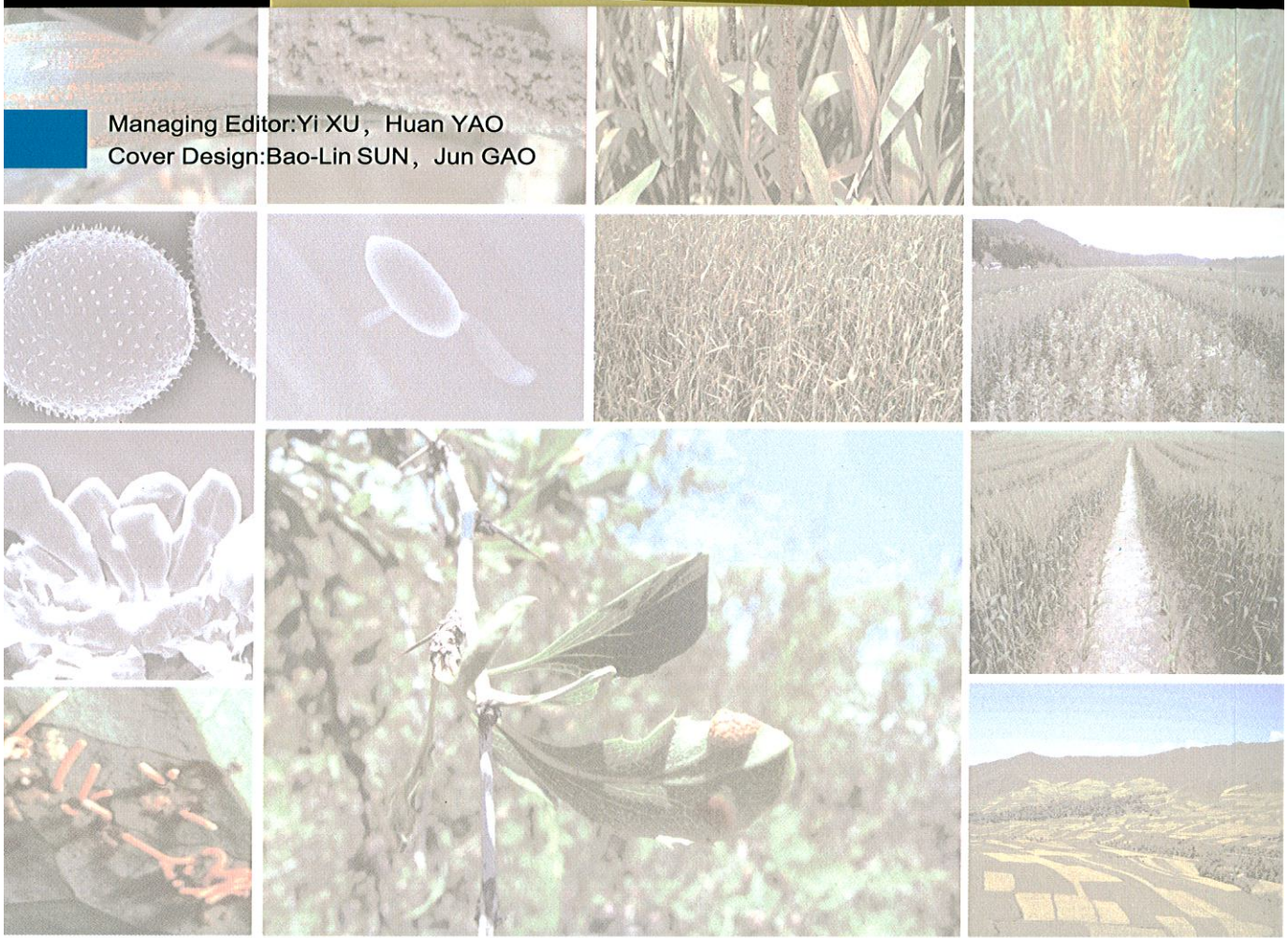
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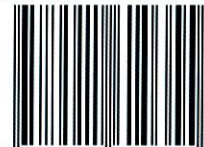


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DIVERSITY OF *Puccinia striiformis* POPULATIONS AT GLOBAL SCALE

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Long-distance migration events at the inter-continental scale have already been reported for *Puccinia striiformis* f. sp. *tritici* (*Pst*). Little is known, however, about the consequences of these migrations on the population structure and the level of diversity in worldwide populations. Here we present results on the population structure and migration footprints of *Pst* based on worldwide samples. Multilocus microsatellite genotyping allowed us to define six genetic groups corresponding to their geographical origin, suggesting the independent maintenance of each population. Clonal population structure was observed in North-West European, Middle-Eastern, Mediterranean, East and South African, and in both North and South American populations. On the other hand, high genotypic diversity and a recombinant population structure was present in three Asiatic populations near Himalayan Chain, i. e. China, Nepal and Pakistan. These Asiatic populations had also a high sex ability assessed by telial production. In addition, pathotype diversity was higher in the Asian groups than in the clonal groups. Our results suggest that the Himalayas represent a centre of diversity for *Pst*, and could be a potential zone of frequent sexual reproduction and thus a probable centre of origin of the yellow/stripe rust fungus.

In the case of European clonal populations, host resistance genes and climate, are considered to be main drivers for shaping the observed *Pst* population structure. At a local scale, in France, the use of specific *Yr* genes explained the virulence spectra of the *Pst* population although some resistances remained durable. Despite long-distance dispersal of *Pst*, two distinct populations were differentiated in North and South of France. Northern strains which had the virulences to develop on southern varieties were not found in the south. South of France has warmer

temperature than North of France and southern isolates were adapted to higher temperature than northern isolates. Local adaptation of southern isolates to high temperature may prevent invasion by northern isolates in south of France. Furthermore, a worldwide expansion of *Pst* into the warm climate regions was shown to be due to the emergence of two aggressive strains adapted to high temperature which have spread since 2000. The information provided on the differential level of diversity at worldwide level and the role of host and climate on the European *Pst* population would help a better *Pst* management in the context of invasion.

THE INFLUENCE OF MUTATION, RECOMBINATION AND
EXOTIC INCURSIONS ON THE RECENT DYNAMICS OF
Puccinia striiformis IN EUROPE

Mogens HOVMØLLER¹, Stephanie WALTER¹, Sajid ALI², Annemarie Fejer JUSTESEN¹, Jens HANSEN¹, Poul LASSEN¹, Rosemary BAYLES³, Kerstin FLATH⁴ and Claude de VALLAVIEILLE-POPE²

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The recent shifts in *P. striiformis* virulence patterns and aggressiveness, which have had a large impact on rust resistance in a number of commercial wheat and triticale varieties in Europe, have stressed the need for timely pathogen survey activities and rapid dissemination of results. Dynamic *P. striiformis* virulence data are now publicly available via the EuroWheat website www.eurowheat.org, which also informs about options for disease control, such as agricultural practices and fungicide efficacies. The emergence of new yellow rust races in the European *P. striiformis* population has until recently been ascribed to frequent mutations resulting in a shift from avirulence to virulence at the phenotypic level, followed by selection due to host resistance genes, clonal reproduction and widespread dispersal of urediniospores carried by the wind at scales from the individual field to continent. However, the appearances of aggressive strains on wheat and triticale, respectively, which have evolutionary origins outside the NW-European *P. striiformis* population, in several European countries, suggest that exotic incursions are playing an increasingly important role. The origin of a new, multivirulent race, which was detected for the first time in 2011 in the UK, France, Germany, Denmark and Sweden, is yet to be determined. However, isolates of this race were often associated with abundant production of telia under both field and green house conditions, which suggest that this new race could have emerged via sexual recombination in Europe or elsewhere.

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**POPULATION STRUCTURE AND TEMPORAL MAINTENANCE
 OF *Puccinia striiformis* IN THE HIMALAYAN REGION
 OF PAKISTAN**

Sajid ALI¹, Pierre GLADIEUX^{2,3}, Hidayatur RAHMAN⁴, Muhammad Shahab SAQIB⁴, Muhammad FIAZ⁵, Habib AHMED⁵, Marc LECONTE¹, Angélique GAUTIER¹, Mogens Støvring HOVMØLLER⁶, Jérôme ENJALBERT¹ and Claude DE VALLAVIEILLE-POPE¹

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Understanding the population structure and temporal maintenance of pathogens in their centre of diversity is crucial to comprehend their evolutionary potential and to assess the risks of future invasions, especially for biotrophic pathogens with long distance migration capacity. Wheat yellow rust pathogen, *Puccinia striiformis* (*Pst*), is a pathogen with long distance migration capacity and a clonal population structure in most parts of the world except at its very recently reported centre of diversity in the Himalayan region. To describe the *Pst* population structure in its centre of diversity, we studied *Pst* populations from the Himalayan region of Pakistan from Khyber to Skardu, sampled in 2010 and 2011 from wheat fields. The microsatellite genotyping of a set of 684 isolates from 14 different locations revealed a high genotypic diversity and recombinant population structure across all locations. Despite the lack of any differentiation in populations across two years (non-significant F_{ST}), only two multilocus genotypes at the maximum were resampled over both years, suggesting a limited across-year survival through clonality. The analyses on population subdivision revealed the existence of spatial population structuring with at least four geographically spaced genetic groups along with some inter-group migrants. A first genetic group dominated Peshawar valley; a second one was prevalent in Siran Valley and resampled in Khyber; the third and fourth groups were present in the other locations, with the third group more

Proceedings of the 13th International Cereal Rusts and Powdery Mildews Conference

prevalent in Haripur. Skardu population from summer-wheat growing region was closely related to the Peshawar valley population, but more diverse, which suggested the existence of some source and sink relationship between winter wheat cropping regions of Peshawar where *Berberis* spp. was absent, and the summer wheat cropping regions of Skardu where *Berberis* spp. was frequent. The pathotyping of a subset of isolates revealed the existence of high pathotypic diversity i. e. , virulences to genes *Yr1*, *Yr2*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *EP*, *A*, *Sd*, *Su*, ad (varieties) Michigan, Victo, Avocet and Cartago were detected in 53 different virulence combinations for 127 isolates tested. Our results suggested a strong influence of sexual recombination in temporal maintenance of *Pst* populations in the Himalayan region of Pakistan, potentially through alternate host (*Berberis* spp.). The presence of very high pathotypic diversity in the region with very high genotypic diversity and sexual reproduction would contribute to pathogen diversity and could serve as a source for new invasions to the rest of the world.

RECENT INVASION OF WORLD-WIDE WHEAT GROWING AREAS BY TWO AGGRESSIVE STRAINS OF *Puccinia striiformis*

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The ever more frequent and severe large-scale epidemics of wheat yellow/stripe rust disease (caused by *Puccinia striiformis*) pose a severe threat to the world's wheat production. The onset of a new series of world-wide wheat yellow rust epidemics in 2000 has been linked to the emergence of two high temperature-adapted aggressive *P. striiformis* strains (named *PstS1* and *PstS2*), first detected in 2000. The origin however of these aggressive strains was unknown. Here we report the development of rapid-to-use SCAR markers for *PstS1* and/or *PstS2* from previously detected AFLP polymorphisms, which uniquely separate these strains from any other *P. striiformis* isolate. The aggressive strain-specific SCARs, as well as 20 SSR markers, were applied on 570 isolates of *P. striiformis* collected from 41 countries on six continents to assess genetic association with strains *PstS1* and *PstS2*. Comparison of the microsatellite profile of these isolates with the previously genotyped worldwide populations suggested the Middle East/Red Sea Area as the origin of the aggressive strains. The combination of SCAR and microsatellite data provided insights to the evolution of these two strains. Based on the marker screening we will provide an update about the global status of the occurrence and virulence spectra of aggressive strains and close relatives.

NONSUPPRESSION OF RESISTANCE IN THE WHEAT THATCHER

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Triticum aestivum Pers. f. sp. *tritici* Eriks. & E. Henn.
in wheat (*Triticum aestivum* L.).
7DL was shown to suppress seedling stem
rust. Seedling resistance in Canthatch was
lost by mutagenesis. The same nonsuppression
was observed when *Lr34*, an adult-plant resistance
gene was introgressed into Canthatch. This demonstrated that
the mechanism of *Lr34* was also shown when 'Thatcher'
genotype carrying *Lr34*. *Lr67* is similar to *Lr34*
for stem rust, and yellow rust. The purpose of this
study was to test the nonsuppression of seedling stem rust
in Thatcher-*Lr34*, Thatcher-*Lr67*, Canthatch,
and Thatcher-*Lr34* × Thatcher-*Lr67* (the suppressor locus deactivated), and
Canthatch-NS1. These genotypes were tested with five different races of
stem rust. Thatcher × Thatcher-*Lr67* was tested with
four races that showed infection types (ITs) that were
reduced in Thatcher-*Lr67*, Canthatch-NS1, and Canthatch-
NS1 and reduced ITs compared to Thatcher and
Thatcher-*Lr67*. For races considered virulent or avirulent was
Thatcher-*Lr67*, Canthatch-NS1, and Canthatch-
NS1. Thatcher-*Lr67* showed lower ITs compared to families that
did not carry *Lr67*. This strongly suggests that *Lr67* has nonsuppressor
activity. We will confirm these findings and to determine if *Lr34*,
and other genes on chromosome 7DL interact with the same loci to
suppress resistance to stem rust.

BARLEY PARTIAL (HOST) AND NONHOST RESISTANCE QTL-NEAR-ISOGENIC LINES AGAINST BARLEY HOMOLOGOUS AND HETEROLOGOUS RUSTS

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Partial (host) and nonhost resistance are possibly associated. Histological studies on partially resistant barley accessions showed that the mechanism of partial resistance to the barley leaf rust fungus (*Puccinia hordei*) is prehaustorial. This is similar to the mechanism of nonhost resistance of barley to heterologous rust fungi, such as the wheat leaf rust fungus (*P. triticea*). This suggests that the two resistance types are based on shared principles, partial resistance resembling a weak form of nonhost resistance. Moreover, QTLs for partial and nonhost resistances of barley tend to co-locate on a consensus map of barley. It is possible that the genes for partial resistance play similar roles in nonhost resistance. Near isogenic lines (NILs) for four partial resistance QTLs (*Rphq2*, *Rphq3*, *Rphq11* and *Rphq16*) and one nonhost resistance QTL (*Rnhq*; L94 allele-*Rnhq.L* and Vada allele-*Rnhq.V*) were developed in SusPtrit genetic background (Sus-QTL). SusPtrit is an experimental line susceptible to several heterologous rust fungi. NILs of *Rphq2*, *Rphq3* and *Rnhq.V* are also available in L94 genetic background (L94-QTL). L94 is somewhat susceptible to a certain number of heterologous rusts. The genome purity of the NILs was evaluated using a barley SNP array, Infinium 9K barley iSelect. Sus-*Rphq16*, L94-*Rphq2* and L94-*Rnhq.V* are clean from the donor genome away from the QTL region. The other NILs have some small introgressions from the undesirable donor genome. The NILs were challenged with different isolates of homologous and heterologous rusts. In general, it is observed that partial resistance QTLs are not only effective towards the homologous rust *P. hordei* but also confer resistance against heterologous rusts. Interestingly, nonhost resistance QTL *Rnhq.V* also confers resistance to the homologous rust *P. hordei* (to one isolate) and to three species of heterologous rusts if in SusPtrit background, but

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not if in L94 background. Differential QTL x isolate interactions also occurred, indicating a minor-gene-for-minor-gene interaction. For some QTLs, the resistance effect in SusPtrit background is different from their effect in L94 background. The results are consistent with the hypothesis that genes for partial resistance to *P. hordei* play also a role in nonhost resistance to heterologous rusts, and that their effectiveness is rust species specific.

IPM STRATEGIES AND THEIR DILEMMAS INCLUDING AN INTRODUCTION TO WWW. EUROWHEAT.ORG

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Information about disease management in winter wheat (*Triticum aestiva*) in eight European countries was collated and analysed by scientists and extension workers within a European Network of Excellence (ENDURE). This included information about specific disease thresholds, decision support systems, host varieties, disease prevalence and pathogen virulence. Major differences in disease prevalence and economic importance were observed. Septoria tritici blotch (*Mycosphaerella graminicola*) was recognized as the most yield reducing disease in countries with intensive wheat production, but also rust diseases (*Puccinia striiformis* and *Puccinia triticina*), powdery mildew (*Blumeria graminis*) and Fusarium head blight (*Fusarium* spp.) were seen as serious disease problems. Examples of current IPM strategies in different countries are reported. Disease management and fungicide use patterns showed major differences with respect to fungicide use, with an average input equivalent to 2.3 full dose rates (TFI) in the UK and a TFI of 0.6 in Denmark. These differences are most likely due to a combination of different cropping systems, climatic differences, disease prevalence and socio-economic factors. The web based information platform www.EuroWheat.org was used for dissemination of information and results including information on control thresholds, cultural practices which can minimize disease attack, fungicide efficacy, fungicide resistance and pathogen virulence, which are all elements which support IPM for disease control in wheat. The platform is open to all users. The target groups of EuroWheat information are researchers, advisors, breeders and similar partners dealing with disease management in wheat.