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Population Biology

e-Xtra*

Genetic Divergence and Diversity in Himalayan *Puccinia striiformis* Populations from Bhutan, Nepal, and Pakistan

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ABSTRACT

The western Himalayan region in Pakistan has been shown to be the center of diversity of *Puccinia striiformis*; however, little is known about its genetic relations with the eastern part of the Himalayas. We studied the genetic structure of *P. striiformis* from Nepal (35 isolates) and Bhutan (31 isolates) in comparison with 81 Pakistani samples collected during 2015 and 2016, through microsatellite genotyping. Genetic analyses revealed a recombinant and highly diverse population structure in Pakistan, Bhutan, and Nepal. A high level of genotypic diversity (>0.90) was observed for the three countries of Pakistan (0.96), Bhutan (0.96), and Nepal (0.91) with the detection of 108 distinct multilocus genotypes (MLGs) in the overall population; 59 for Pakistan, 27 for Bhutan, and 26 for Nepal. Mean number of alleles per locus and gene diversity were higher in Nepal (3.19 and 0.458, respectively) than Bhutan (3.12 and 0.458, respectively). A nonsignificant difference between the observed and the expected heterozygosity in all populations further confirmed the recombinant structure. A clear population subdivision between the Himalayan region of Nepal, Bhutan, and Pakistan was evident, as revealed by F_{ST} values (ranging between 0.111 to 0.198), discriminant analysis of principal components, and resampling of MLGs. Limited gene flow could be present between Nepal and Bhutan, while the population from Pakistan was clearly distinct, and no divergence was present between two populations from Pakistan (Bajaur and Malakand). The overall high diversity and recombination signature suggested the potential role of recombination in the eastern Himalayan region (Nepal and Bhutan), which needs to be considered during host resistance deployment and in the context of aerial dispersal of the pathogen. Further surveillance should be made in the Himalayan region for disease management in the region and in the context of worldwide invasions.

Keywords: diversity, divergence, population genetics, stripe rust, simple sequence repeat genotyping

Food security in developing countries is always challenging, where many factors affect the continuous and sufficient supply of food including devastating epidemics of diseases of food crops. Wheat, an important crop ensuring food security in many developing countries, is continuously facing environmental threats and disease risks. Among diseases, rust diseases particularly yellow rust (caused by *Puccinia striiformis*), pose severe threat to higher yield especially in the areas where the mild cold climate prevails during wheat cropping season (Chen 2005; Hovmøller et al. 2011). The pathogen survives better in areas where there are moist and cool weather conditions. The geographical range of *P. striiformis* is worldwide; it has occurred throughout recent history in Asia, Europe, and North Africa and in the early 1900s it was introduced into the Americas, and more recently into Australia and South Africa (Ali et al. 2014a; Beddow et al. 2015; Hodson 2011; Hovmøller et al. 2010). The onset of disease on susceptible host in favorable environment leads to significant economic losses worldwide (Ali et al. 2017a).

To cope with the disease of wheat yellow rust, the use of chemicals has been suggested, which however, is not affordable to farmers of developing countries where the use of genetic resistance strategy is more fruitful and affordable (Ali et al. 2009; de Vallavieille-Pope et al. 2012; Paillard et al. 2012; Pathan and Park 2007). Genetic resistance is more economic, without any health and environment hazards (Chen 2005). Despite continuous efforts to develop resistant wheat varieties through incorporation of resistance genes, the effectiveness of these resistance genes remains limited due to acquisition of virulence by the pathogen population (de Vallavieille-Pope et al. 2012). More than 53 yellow rust resistance genes have been identified and many of these incorporated into wheat varieties (McIntosh et al. 2010). When resistant varieties, especially those with single major gene resistance, are deployed over large geographical regions there is high selection pressure for virulent variants of the pathogen. Virulent variants (either strains or lineages) of *P. striiformis* thus emerge to infect the resistant host varieties deployed over large areas.

Virulent strains could either evolve locally or through invasion from one location to another via wind or human-borne movement (Halkett et al. 2005; Hovmøller and Henriksen 2008; Hovmøller et al. 2002; Markell and Milus 2008; Wellings and McIntosh 1990). Local evolution occurs either through mutation or recombination. For recombination, rust fungi require both the primary and alternate hosts. The alternate hosts for yellow rust fungi are *Berberis* and *Mahonia* species (Jin et al. 2010; Rodriguez-Algaba et al. 2014). With sexual recombination on the alternate host, the pathogen can cope with adverse environmental conditions, provide inoculum to infect cereals every year, and generate new races resulting in overall

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diversification of the pathogen population (Taylor and Gurr 2014). Exotic incursions can also be a source of new variants. The pathogen is highly mobile, either through the movement of people, trade intensification, or aerial dispersal. *P. striiformis* has the capability to rapidly spread to new regions and crop varieties. For example, in 1979 wheat yellow rust was spread to Australia from Europe through human travel (O'Brien et al. 1980). The pathogen dispersal in the United Kingdom, Denmark, Germany, and France caused yellow rust on wheat varieties that were previously known to be resistant to the pathogen (Hovmøller et al. 2002). Thus, efforts for disease monitoring and control should be concentrated not only at the country level, but across the adjacent populations, which could serve as sources of invasions.

Recently, new emerging strains have been associated with worldwide wheat yellow rust epidemics (Ali et al. 2017a), including lineages aggressive at relatively warm climatic conditions (Hovmøller et al. 2008, 2016; Milus et al. 2009). A number of these lineages have been postulated to have originated in the near-Himalayan region (Hovmøller et al. 2016; Ali et al. 2017a). These near-Himalayan populations have been shown to be recombinant and highly diverse (Ali et al. 2014a, 2016; Thach et al. 2016), in contrast to the clonal population structure in the Americas, Europe, and Australia (Ali et al. 2014a). New variants arising in the Himalayan center of diversity can pose a threat to other wheat regions through aerial dispersal or human mediated transfer. Considering the importance of this pathogen dispersal threat, the population structure in the Himalayan regions of Pakistan, Nepal, and Bhutan needs to be studied and monitored in a worldwide context. Yellow rust has been, and continues to be, important in Pakistan, Nepal, and Bhutan (Bux et al. 2012; Mann and Hobbs 1988; Singh et al. 2004). However, the population structure of *P. striiformis* is unknown in Bhutan and limited information is available for Nepal, particularly in relation to the western Himalayan populations of Pakistan.

The present study was designed to understand *P. striiformis* population structure in the eastern Himalayan regions of Nepal and Bhutan along with the Pakistani Himalayan region based on samples collected in 2015 and 2016. The specific objectives of this study were to investigate diversity and signature of recombination presence of any population subdivision in *P. striiformis* populations from Pakistan, Nepal, and Bhutan, and to compare the population structure of Nepalese and Bhutanese Himalayan population with the Pakistani Himalayan population of *P. striiformis*.

MATERIALS AND METHODS

Sample collection and single lesion preparation. To describe the population structure of *P. striiformis* present in the Himalayan region of Nepal, Bhutan, and Pakistan, we collected a set of 147 samples from field infected wheat plants in 2015 and 2016 (Supplementary Fig. S1). This consisted of 81 single lesion samples from Pakistan (44 from Bajaur district and 37 from Malakand district), 31 from Bhutan, and 35 from Nepal (Supplementary Table S1). Single lesions were excised from these samples in situ for subsequent DNA extraction as described by Ali and Hodson (2017).

TABLE 1. Number of alleles, Simpson's diversity index, expected heterozygosity, and evenness calculated for 16 simple sequence repeat (SSR) loci in 66 *Puccinia striiformis* isolates sampled from Bhutan and Nepal

SSR locus	Number of alleles detected	Simpson's diversity index (1-D)	Gene diversity	Evenness
RJO4	3	0.620	0.623	0.905
RJO24	8	0.801	0.803	0.833
RJN12	5	0.093	0.094	0.396
RJN8	3	0.086	0.086	0.436
RJN13	2	0.066	0.066	0.440
RJN11	7	0.761	0.764	0.847
RJN6	4	0.250	0.251	0.498
RJO21	4	0.343	0.345	0.562
RJN10	3	0.526	0.528	0.886
RJO18	7	0.630	0.632	0.756
WU6	5	0.195	0.196	0.435
RJO20	4	0.548	0.550	0.785
RJN4	4	0.554	0.556	0.854
RJN9	4	0.029	0.029	0.311
RJN5	5	0.622	0.624	0.774
WU12	4	0.698	0.700	0.899

DNA extraction and microsatellite genotyping. DNA was extracted directly from single lesions (Ali et al. 2011a, b) using modified CTAB method (Ali et al. 2017b). The extracted DNA was amplified using 16 fluorescent simple sequence repeat (SSR) markers in two PCR multiplexes (Ali et al. 2017b). Fragment analysis of the amplified product was accomplished using the services of Uppsala University Genome Center through Aarhus University, Denmark. Fragment readings were carried out with GENEMARKER 4.05.2 software, reading each allele based on its allele size and formatted in MS Excel file (Ali et al. 2014b).

Population genetic analyses and interpretation. The suitability of markers to determine multilocus genotypes (MLGs) was assessed by plotting the number of MLGs detected versus the number of loci. GENETIX program was used for the estimation of F_{ST} to measure population subdivision. Spatial population structure was further assessed using both model-based Bayesian and non-parametric multivariate clustering methods. Model-based Bayesian method implemented in the STRUCTURE 2.2 software (Pritchard et al. 2000) was used to assign MLGs into clusters with 20 independent runs, which were processed with CLUMPP (Jakobsson and Rosenberg 2007) to assign groups of runs to a common clustering pattern (G'-statistic greater than 80%). The optimal K value was determined using the method of Evanno et al. (2005) based on the rate of change in the log probability of data between successive K values. The results were further confirmed with discriminant analysis of principal components (DAPC) carried out in the ADEGENET package (Jombart et al. 2010). Phylogenetic tree was constructed using POPULATION software (Langella 2008) based on the genetic distances (Nei) between genotypes from the SSR dataset. Principal coordinate analysis of a Euclidean distance matrix was done in ADE4 package for both geographically spaced populations and STRUCTURE identified genetic groups.

The summary statistics were calculated using POPPR package within R-software. The summary statistics included number of different MLGs (showing genotypic richness); mean number of alleles and gene diversity (showing genetic diversity); Simpson's diversity index (showing genotypic diversity); evenness index (reflecting on relative distribution of genotypes abundance); and standardized index of association (rDbar) and its significance, showing linkage over loci. GENETIX 4.05.2 software (Belkhir et al. 2004) was used for the assessment of recombination signature by estimating the difference between the observed heterozygosity (Ho) and the expected heterozygosity (He).

RESULTS

Summary statistics of microsatellite markers. All 147 isolates of *P. striiformis* samples showed a varied level of polymorphism over the studied loci (Table 1). The number of alleles per locus ranged from two for RJN13 to eight for RJO24. The expected heterozygosity across loci was found to vary between 0.029 (RJN9) and 0.801 (RJO24). The evenness across loci ranged from 0.311 (RJN9) to 0.905 (RJO4). The tested markers were enough to capture the maximum diversity in the population as revealed by plotting the identified MLGs against the number of loci (Supplementary Fig. S2). Increasing the number of loci resulted in increasing the capacity of markers to capture the MLGs. The maximum number of MLGs (108) were already captured by 13 loci, reaching the maximum distribution around 108 MLGs at 16 loci.

Genetic diversity and recombination signature. High diversity and a recombinant population structure were observed in the *P. striiformis* populations from Nepal, Bhutan, and Pakistan as assessed through estimation of the expected heterozygosity and allele richness. There was a nonsignificant difference between the expected heterozygosity (He) and observed heterozygosity (Ho) for Nepalese, Bhutanese, and Pakistani (Malakand and Bajaur) *P. striiformis* populations suggesting recombination in all studied locations (Fig. 1). Gene diversity in the overall population was found to be 0.428. Gene diversity was higher in Nepalese population (0.458) followed by Malakand (0.368), Bajaur (0.348), and Bhutanese population (0.344). Overall, a high genotypic diversity was observed in all populations as revealed by Simpson's index (0.957 for Bhutan and Malakand; Table 2) compared with the clonal populations in Europe where the diversity within the crop season was less than 0.50 (Ali et al. 2014a; Thach et al. 2016). The high diversity was further affirmed by the 108 distinct MLGs (out of 147 samples) detected in the overall population, out of which 27 were detected in Bhutan, 26 in Nepal, 29 in Bajaur, and 30 in Malakand regions from Pakistan (Table 2).

Population subdivision in the Himalayan population. The principle coordinate analysis revealed three groups, one specific to Bhutan, another one in Nepal, and the third one predominantly present in Pakistan, with some resampling in Bhutan and Nepal and little in Pakistan as well (Fig. 2A). This population subdivision was further confirmed by a highly significant ($P < 0.001$) and strong F_{ST} value of geographically different populations ranging from 0.196 to 0.168, while the F_{ST} among Bajaur and Malakand was estimated to be 0.032 (Table 3). Neighbor joining tree and network analyses further confirmed the population subdivision across countries, where samples from Bhutan were relatively closer to the Nepalese, with limited closeness to Pakistani samples (Fig. 2B and C).

To identify genetic clusters in the tested populations and assignment of samples into these clusters, the model-based STRUCTURE and DAPC analyses suggested the existence of at least four clusters in the data (Supplementary Fig. S3), which were quite distant from each other when plotted over a scatter plot (Fig. 3; Supplementary Fig. S4). Assignment of isolates into these clusters revealed that one cluster was specific to Bhutan only (G4) with limited admixture in Pakistan, while one cluster (G3) was predominant in Nepal but resampled in Pakistan and Bhutan as well. G1 was predominant in Pakistan, though with limited admixture in Bhutan as well (Fig. 3). G2 was specific to the eastern Himalayas, present only in Bhutan and Nepal, and absent in Pakistan. An overall high diversity and signature of recombination was observed within each genetic group identified with STRUCTURE (Table 4) and DAPC (Supplementary Table S3).

We assessed the placement of worldwide predominant linages (PstS0 till PstS10; Ali et al. 2017a) in comparison with these Himalayan population through making a neighbor-joining tree based on comparison of SSR profile of these worldwide linages (available from Ali et al. 2017a). The lineages PstS6, PstS7, and PstS9 were placed within the Himalayan populations, as reported in the worldwide study as well (Ali et al. 2017a). The rest emerged as separate linages (Fig. 4).

Resampling of MLGs and their spatial distribution. Resampling of MLGs at the same location or across different geographically spaced populations reflected the presence of low clonality and very limited migration of clones from one region to the other. A limited number of MLGs were resampled two or more than two times. None of the MLGs were found to be present across all the geographically spaced populations. Out of 108 distinct MLGs, only 15 were resampled. Only two MLGs were resampled between Nepal and Bhutan, i.e., MLG-71 was resampled 12 times (nine times in Nepal and three times in Bhutan) and MLG-81 sampled once in Nepal and once in Bhutan. None of the MLGs were shared between Pakistan and either Nepal or Bhutan. In Pakistan, MLG-51 and MLG-52 were resampled six times in Bajaur only. Similarly, three MLGs were resampled four times and nine MLGs were resampled only twice at the same or different location (Table 5). This is further evidenced from the haplotype network analyses (Fig. 2C).

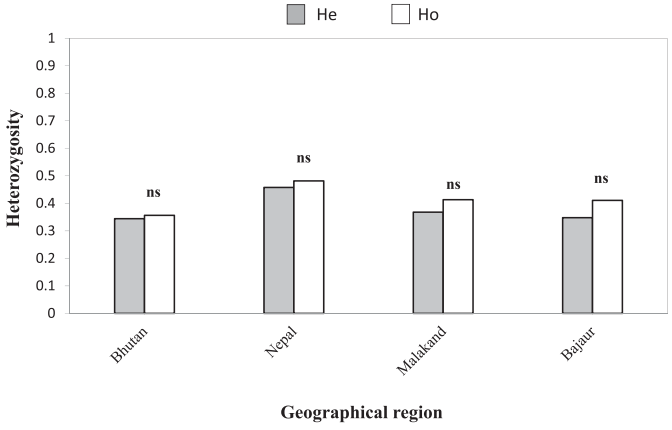

Fig. 1. Observed (Ho) and unbiased expected (He) heterozygosity over 16 microsatellite markers for *Puccinia striiformis* populations sampled in Bhutan, Nepal, and Pakistan (Bajaur and Malakand).

DISCUSSION

This study describes the genetic structure of the *P. striiformis* population in the Himalayan region of Pakistan, Nepal, and Bhutan. Based on a set of 147 field samples (35 from Nepal, 31 from Bhutan, and 81 from Pakistan) from 2015 to 2016. Microsatellite genotyping and subsequent population genetics analyses revealed a high diversity and recombinant population structure in the eastern Himalayan region with limited migration across countries. Results would support recent research indicating the Himalayan region as a center of diversity for *P. striiformis* (Ali et al. 2014a).

Recombination signals and high genetic diversity in both eastern and western Himalayas. High genetic diversity and strong signal of recombinant population structure was evident in the eastern Himalaya region (Nepal and Bhutan) and in the western Himalayan region (Pakistan). High values of diversity indices, low linkage disequilibrium and lack of difference between expected and observed heterozygosity supported this proposition. Previously, the

TABLE 2. Diversity parameters in *Puccinia striiformis* populations sampled in the Himalayan region of Bhutan, Nepal, and Pakistan (Bajaur and Malakand)

Diversity parameters	Bhutan	Nepal	Bajaur	Malakand	Overall population
Sample size	31	35	44	37	147
Number of different multilocus genotypes	27	26	29	30	108
Mean number of alleles	3.125	3.188	3.500	2.688	3.125
Simpson's diversity index	0.957	0.911	0.940	0.957	0.982
Evenness index	0.916	0.565	0.720	0.853	0.642
Gene diversity	0.344	0.458	0.348	0.368	0.428
Standardized index of association (rDbar)	0.132	0.273	0.198	0.088	0.132
P value for rDbar	0.001	0.001	0.001	0.001	0.001

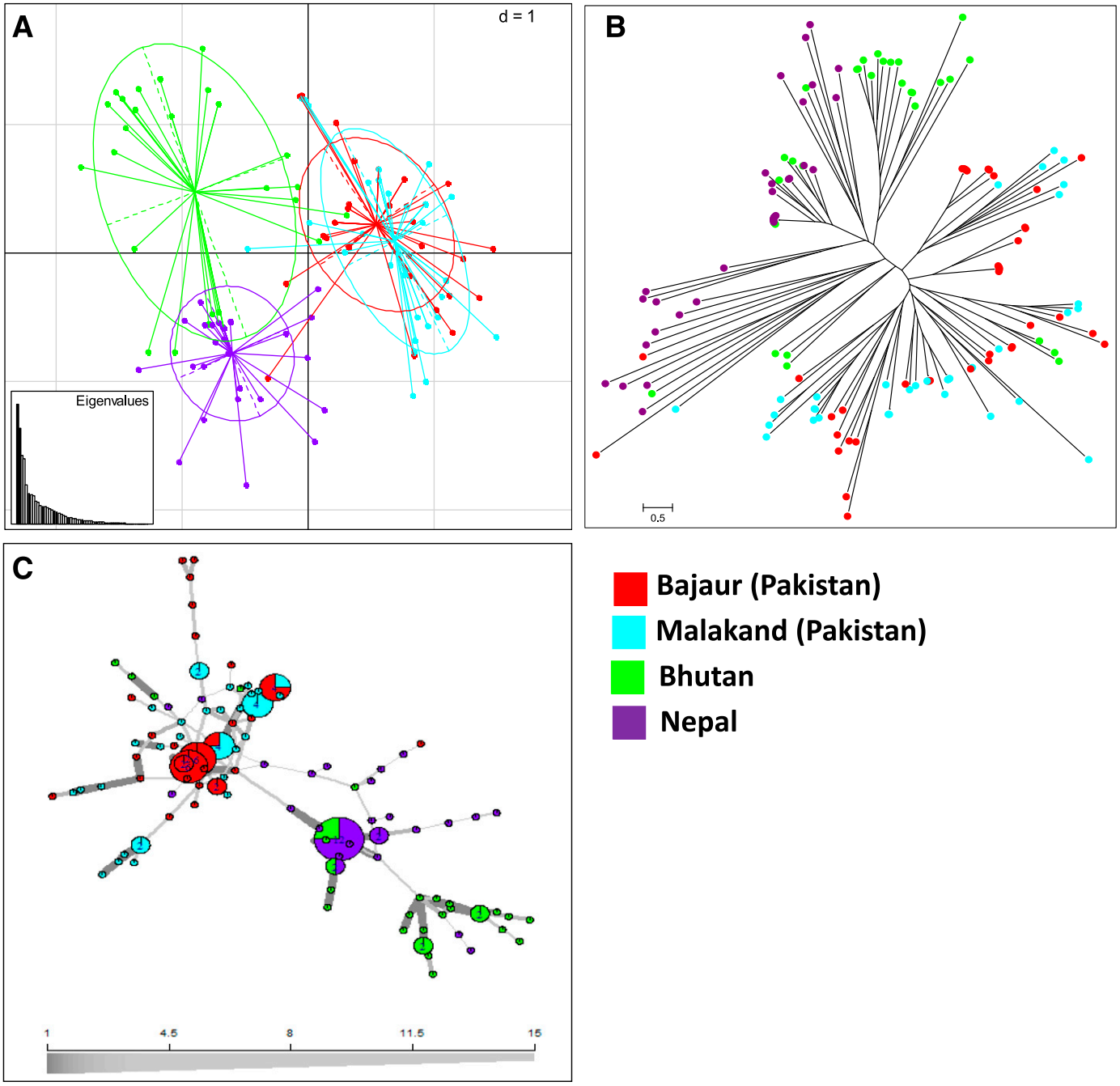

Fig. 2. Relationship of Himalayan *Puccinia striiformis* populations from Bhutan, Nepal, and Pakistan (Bajaur and Malakand) based on 16 microsatellite genotype data as revealed through **A,** principal coordinate analysis, **B,** neighbor-joining tree, and **C,** network analyses.

TABLE 3. Divergence (in terms of F_{ST} values, upper diagonal and its significance, the lower diagonal) among the geographical spaced populations and STRUCTURE based genetic groups of *Puccinia striiformis* populations sampled in the Himalayan region of Bhutan, Nepal, and Pakistan (Bajaur and Malakand)

	Population	Bhutan	Nepal	Malakand	Bajaur
Geographical spaced populations	Bhutan	–	0.111	0.196	0.191
	Nepal	0.000	–	0.168	0.179
	Malakand	0.000	0.000	–	0.032
	Bajaur	0.000	0.000	0.000	–
STRUCTURE based genetic groups	Group	G1	G2	G3	G4
	G1	–	0.238	0.201	0.286
	G2	0.000	–	0.166	0.310
	G3	0.000	0.000	–	0.278
		0.000	0.000	0.000	–

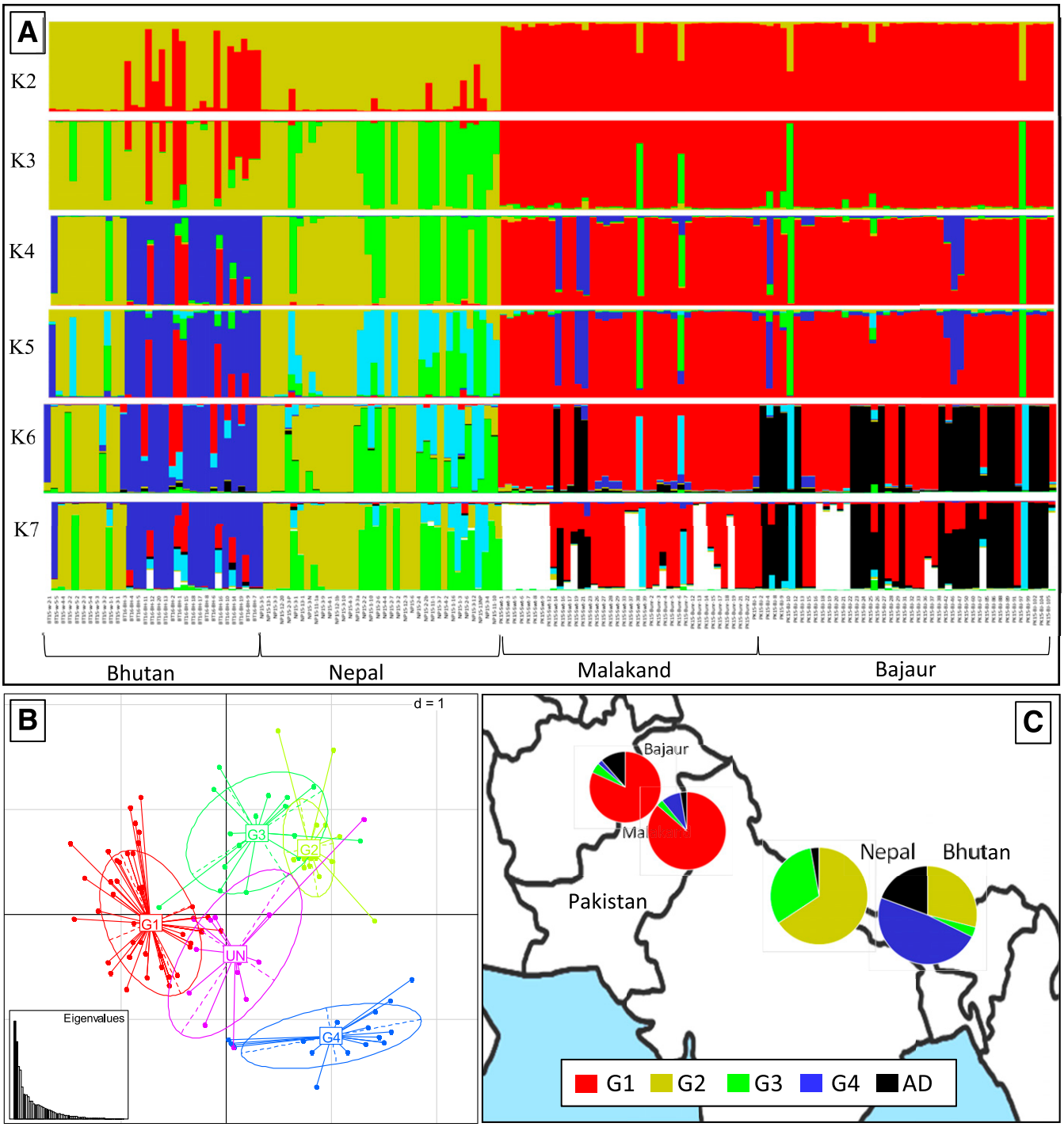

Fig. 3. Structure-based assignment of *Puccinia striiformis* samples from Bhutan, Nepal, and Pakistan. **A,** Assignment of samples into various clusters (K2-7). **B,** Scatter plot of the clusters identified at K=4. **C,** Distribution of the four clusters and admixed group across the Himalayan populations.

TABLE 4. Diversity parameters for genetic groups identified through STRUCTURE software in the *Puccinia striiformis* populations from the Himalayan region of Bhutan, Nepal, and Pakistan

Diversity parameters	G1	G2	G3	G4	Admixed
Sample size	68	31	16	19	13
Number of different multilocus genotypes	44	18	16	17	13
Mean number of alleles	2.625	2.125	3.937	2.062	3.000
Simpson's index	0.962	0.826	0.938	0.936	0.923
Evenness index	0.751	0.483	1.000	0.953	1.000
Gene diversity	0.119	0.240	0.049	0.103	0.106
Standardized index of association (rDbar)	0.060	0.140	0.049	0.099	0.106
P value for rDbar	0.001	0.001	0.001	0.001	0.001

Himalayan and near-Himalayan regions including Pakistan, Nepal, and China were reported to be the center of origin and diversity for *P. striiformis* (Ali et al. 2014a), where the pathogen was postulated to undergo sexual reproduction on *Berberis* spp. (Ali et al. 2014b; Jin et al. 2010; Ray et al. 2011). Similarly, the ancestral relationship also confirmed the Himalayan population to be the ancestral population (Ali et al. 2014a). The observed high genetic diversity was in contrast to other areas of the world, where the genetic variability of *P. striiformis* population was found to be very low (Ali 2012; Ali et al. 2014a). A clonal population structure was observed in United States (Chen 2005), Australia (Thach et al. 2016), and Europe (Ali et al. 2014a; de Vallavieille-Pope et al. 2012; Thach et al. 2016). Observed genetic diversity was also higher than the Middle Eastern and Mediterranean populations (Ali et al. 2014a; Bahri et al. 2009).

The observed high genotypic diversity would also imply high adaptation capacity of the pathogen to acquire diverse virulence profiles and adapt to diverse environmental conditions (Ali et al. 2010; Jin et al. 2010). A high diversity in virulence and pathotype was previously observed in recombinant populations of Pakistan (Ali et al. 2014c; Ali et al. 2017a). Little was known about the virulence and phenotype structure of *P. striiformis* in Nepal, with only a recent study showing low pathotype diversity with high genetic diversity (Ali et al. 2018). No published study on the Bhutanese *P. striiformis* population and the relationship between these Himalayan populations is available to date, whereas only a limited number of *P. striiformis* samples from Bhutan have been pathotyped (Ali et al. 2017a). Initial results indicated potential diversity. Hovmøller et al. (2016) reported six races from 10 isolates. Considering the high genetic diversity in both Nepal and Bhutan, expanded analysis on virulence and pathotypes must be made in these regions. Populations containing more variations in alleles could provide variation suited to the environment into which they are migrating, thus enabling successful invasions from the diverse populations (Hovmøller et al. 2016).

Population subdivision and migrations within Himalayan *P. striiformis* populations. Analysis revealed a clear population subdivision between the Himalayan region of Nepal, Bhutan, and Pakistan, as revealed by F_{ST} values, DAPC analyses, and resampling of MLGs. Though a very limited gene flow could be present between Nepal and Bhutan, the population from Pakistan was clearly distinct. Samples representing various genetic groups in a country may have different adaptation to host resistance and may pose a threat if migration occurs from one lineage prevalent region

TABLE 5. Distribution of distinct multilocus genotypes (MLGs) detected in *Puccinia striiformis* populations sampled in the Himalayan region of Bhutan, Nepal, and Pakistan (Bajaur and Malakand)

MLG	Bhutan	Nepal	Malakand	Bajaur	Overall population
MLG-71	3	9	–	–	12
MLG-51	–	–	–	6	6
MLG-52	–	–	–	6	6
MLG-40	–	–	4	–	4
MLG-41	–	–	1	3	4
MLG-42	–	–	3	1	4
MLG-102	2	–	–	–	2
MLG-103	2	–	–	–	2
MLG-24	–	–	2	–	2
MLG-50	–	–	–	2	2
MLG-53	–	–	–	2	2
MLG-6	–	–	2	–	2
MLG-67	–	2	–	–	2
MLG-81	1	1	–	–	2
MLG-95	–	–	–	2	2
Number of single MLGs	23	23	25	22	93
Sample size	31	35	37	44	147

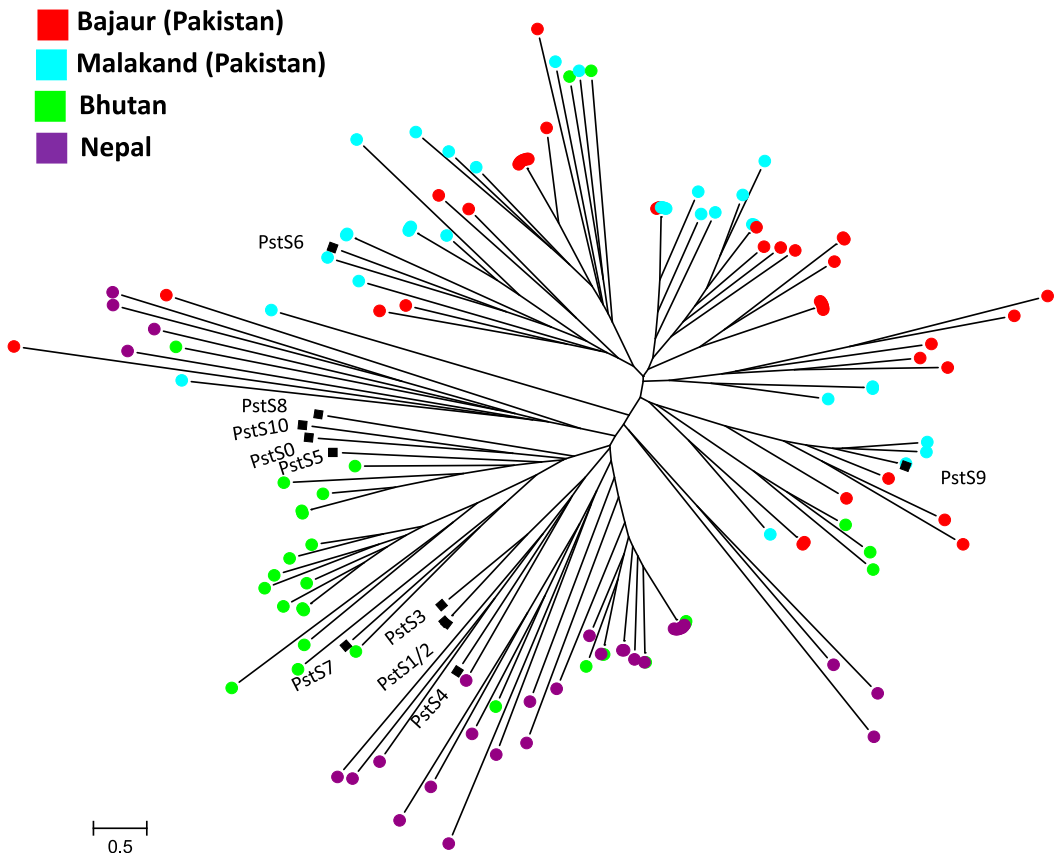

Fig. 4. Placement of worldwide predominant linages (PstS0 till PstS10; Ali et al. 2017a) in comparison with these Himalayan populations through a neighbor-joining tree based on comparison of simple sequence repeat profiles of these worldwide linages (available from Ali et al. 2017a).

to another (Hovmøller et al. 2016; Walter et al. 2016). *P. striiformis* is well known for its long-distance migration capacity through wind dispersal (Brown and Hovmøller 2002), and thus a limited gene flow over the region could still be possible. Thus, the existence of overall population subdivision could be explained by the local maintenance of the population with a potential role of recombination in each country (Ali et al. 2016) isolated by high mountainous ranges. Finally, the wheat varieties grown in the region are predominantly CIMMYT-based varieties (Lantican et al. 2016), which are often genetically related across the region and include identical varieties released in multiple countries (Ali et al. 2014c; Singh et al. 2004). Indeed, the role of host selection has been limited in the Himalayan region, where multiple linages can infect the same variety (Khan and Ali 2018) and the same pathotype can be represented by distinct MLGs (Ali et al. 2017a, 2018).

Implication for worldwide yellow rust management. Yellow rust is expanding its range globally (Beddow et al. 2015) with both wind-borne and human-mediated transport by important dispersal mechanisms (Brown and Hovmøller 2002; O'Brien et al. 1980). Recent, long-distance exotic incursions, e.g., the 'Warrior' (PstS7) race into Western Europe (Hovmøller et al. 2016) and race PstS11 from Afghanistan into East Africa (Hovmøller et al. 2018), highlight the importance of the Himalayan/near-Himalayan region as a source of new virulence profiles that result in damaging consequences in distant regions. Invasive strains originating in diverse populations could have advantage over the existing pathogen populations if they harbor novel virulence factors and/or adaptation to new climatic conditions (Walter et al. 2016). It could also be due to increased aggressiveness of the invasive strains on the hosts, which were considered to be resistant to the prevailing pathogen populations (Hovmøller et al. 2016). Invasive strains with novel virulence profiles, adaptation to new climatic conditions, or increased aggressiveness can rapidly replace prevailing pathogen populations, resulting in damaging epidemics over large geographical regions (Ali et al. 2017a; Walter et al. 2016). Results of this study highlight the critical need for expanded monitoring and understanding of populations in the Himalayan region, coupled to improving collaborative efforts to manage yellow rust at the national, regional, and global scale.

Conclusion. Our study revealed a strong signal for a recombinant population structure in Pakistan, Bhutan, and Nepal with very high genetic diversity, but limited migration across the countries. The results imply that there are most likely multiple sources of *P. striiformis* variation occurring within the Himalayan region. Given the high dispersal capacity of *P. striiformis*, these sources of new variation and diversity have global importance. Previously, invasive strains of *P. striiformis* introduced into new regions has resulted in huge epidemics across large geographical regions. To avoid such surprises and emergency situations, collaborative efforts has strongly been suggested to tackle the disease at the national, regional, and worldwide scale (Ali et al. 2017a).

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