# Effect of Genetic and Phenotypic Variation in *Puccinia striiformis* on the epidemiology of yellow rust in Sweden (V1133036)

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## Background

Yellow rust or stripe rust (caused by *Puccinia striiformis*) was traditionally known as a rust that was favoured by cooler, wetter weather. Unlike many other rusts on small grains, it was not known to have an alternate host, and it was thought to overwinter on the grain host (wheat, rye, barley, triticale) forming clonal lineages over wide geographic areas. While resistance genes are available in different wheat cultivars, these are frequently overcome by new races of the pathogen that render these resistance genes useless for the control of disease initiated by such races. Since sexual reproduction was not known for this fungus, these new races were thought to develop mainly via mutation, or possibly by somatic recombination, although this has not yet been confirmed to take place in an agricultural cropping situation.

In recent years, new information about the epidemiology of yellow rust has become available. Variants of the pathogen developed that were able to grow in warmer areas that were thought to be outside the domain of yellow rust, and one of these became widespread in major wheat growing areas around the world (Brown, 2002; Hovmøller *et al.*, 2008). Population studies of *P. striiformis* with molecular markers has shown that there was considerable variation of the pathogen in China (Mboup *et al.*, 2009), but much less in Europe and North America. Studies have been done with both AFLP and microsatellite markers (Enjalbert *et al.*, 2002; Justesen *et al.*, 2002; Chen *et al.*, 2009) and efforts to sequence the pathogen will undoubtedly lead to the availability of additional markers, such as single nucleotide polymorphisms (SNPs). The use of these markers is essential in understanding the population biology of the pathogen, since only they can be used to establish the genetic relatedness of different isolates.

More recently, scientists at the Cereal Disease Laboratory in the US have shown that several barberry species (*Berberis* spp.) may serve as the alternate host of *P. striiformis* (Jin *et al.*, 2010). Sexual reproduction may explain the increased genetic variation in certain areas, and may also be a factor in the development of new races. A full sexual cycle could also mean that barberry plants could serve as sources of initial inoculum for crop epidemics, just as they function as sources of *Puccinia graminis*, which causes stem rust. The teliospores of *P. striiformis* do not seem to require winter conditions to promote germination, which means that this could take place in the fall and not only in the spring.

In Sweden, yellow rust is a problem primarily in triticale and wheat, and to a lesser extent in barley. Since no alternate host was previously known, it was thought to overwinter only on the grain host. Since mild winters favour the overwintering of the pathogen, yellow rust was primarily a problem in southern Sweden. More recently, though, yellow rust has been reported as far north as Mälardalen. Whether this change in the geographic distribution of yellow rust is due to a warmer climate or changes in the pathogen is not known. A limited number of samples of yellow rust from Sweden are being analyzed for race composition, and at present it appears that one race dominates on wheat (commonly found on cv. Tulsa) and another is dominant on triticale (infecting e.g. cvs. Dinaro and Cando). This “Tulsa” race may also lie behind the increased yellow rust problems on other cultivars of wheat, such as Olivin. Analysis of this wheat race, with molecular markers, indicates that it is not directly derived from any of the common clonal lineages in Northern Europe but it originates from Asia (Ali *et al.*, 2014).

It is not known if sexual reproduction of *P. striiformis* takes place in Sweden, and if it plays any role in the epidemiology of the disease. Studies at Aarhus University have confirmed the ability of *P. striiformis* from Denmark to undergo the full sexual cycle (Rodriguez-Algaba *et al.*, 2014). As part of a study of stem rust in oats, we have collected aecia from barberry plants. This species has become quite common since the repeal of the barberry eradication law in 1994, and aecia are commonly found on these plants in the spring. Analysis of the ITS region of these samples shows that the majority of them are *P. graminis*, but a limited number of samples have shown high correlation to *P. striiformis*, with matching of up to 97%. Variation in the ITS region exists for the rust fungi collectively known as *P. graminis* (Abbasi *et al.*, 2005), and if this is the case for *P. striiformis*, one cannot exclude the possibility that sexual reproduction of yellow rust takes place in Sweden.

## Materials and Methods

Sampling of rust was made from wheat and triticale fields in Sweden, including variety trials, in 2012 and 2015. In 2012, rust was sampled from fields in Gotland, Kalmar area, Skåne, Uppsala area, Östergötland, and Västergötland, and processed for genetic analyses. In addition, 60 samples consisting of aecia were taken in 2012, primarily in Östergötland and Kalmar area. In 2015, samples were taken from a series of field trials, and samples were takaen from winter wheat trials in Uppåkra and Klagstorp, spring wheat in Uppåkra, and a triticale trial in Rydsgård, all of them in Skåne, and a spring wheat trial on Öland. A total of 60 aecia were also taken in 2012, and their identity was checked by sequencing the ITS region (Berlin *et al.*, 2013a).

Both genetic (with microsatellites) and phenotypic (with differential cultivars) analyses were planned for this material, but the phenotypic analyses were only possible on the 2015 material due to staffing difficulties. For the genetic studies, single pustules from wheat and uredinia were removed from the leaves, homogenized, and the DNA extracted with an OmniPrep kit (Berlin *et al.*, 2013b; Nilsson, 2016). Primers and a protocol for sets of microsatellite markers (Ali, 2011) were used to produce DNA that could be sized for genetic studies. Data was analysed with the programs poppr (Kamvar *et al.*, 2014) and structure (Pritchard *et al.*, 2000).

Samples from 2015 were also used for race analyses at the Global Rust Reference Centre. Single pustule isolates were first made, and then sufficient inoculum was produced to inoculate wheat plants with resistance genes, and the reactions were then scored. More details for the procedure for these analyses can be found in Nilsson (2016).

## Results

For the samples from 2012, a total of 330 samples from Sweden produced usable DNA. In addition, 23 additional samples were taken from neighbouring Baltic countries and Norway. This gave 353 samples that could be analysed with 18 microsatellite markers (Table 1).

Some of the microsatellite markers performed relatively poorly and 3 loci with more than 50% missing were eliminated from the data set. This was still insufficient for the next data analyses, so 69 genotypes were also eliminated if they had more than 50% of the loci missing. This gave a total of 284 individuals for this final analysis.

| Pop | N | MLG | eMLG | SE | H | G | Hexp | E.5 | Ia | rbarD |
|---|---|---|---|---|---|---|---|---|---|---|
| Gotland | 88 | 66 | 9.57 | 0.64 | 4.05 | 46.7 | 0.99 | 0.81 | 4.24 | 0.341 |
| Kalmar | 68 | 61 | 9.71 | 0.56 | 4.02 | 45.3 | 0.993 | 0.81 | 1.36 | 0.109 |
| Skane | 60 | 48 | 9.21 | 0.89 | 3.66 | 25.4 | 0.977 | 0.641 | 2.42 | 0.201 |
| Uppsala | 50 | 40 | 9.15 | 0.91 | 3.5 | 22.3 | 0.975 | 0.667 | 1.73 | 0.173 |
| Västergötland | 21 | 17 | 8.83 | 0.88 | 2.71 | 12.6 | 0.967 | 0.823 | 2.14 | 0.212 |
| Östergötland | 43 | 26 | 8.41 | 1.05 | 3.01 | 15.5 | 0.958 | 0.757 | 1.92 | 0.24 |
| Latvia | 3 | 2 | 2 | 0 | 0.64 | 1.8 | 0.667 | 0.899 | NaN | NaN |
| Estonia | 8 | 8 | 8 | 0 | 2.08 | 8 | 1 | 1 | 1.68 | 0.209 |
| Norway | 6 | 6 | 6 | 0 | 1.79 | 6 | 1 | 1 | 2.21 | 0.278 |
| Finland | 6 | 6 | 6 | 0 | 1.79 | 6 | 1 | 1 | 0 | NaN |
| Total | 353 | 262 | 9.79 | 0.46 | 5.33 | 129 | 0.995 | 0.623 | 2.44 | 0.183 |

Table 1. Poppr analysis of the initial 353 samples from 2012.

| Pop | N | MLG | eMLG | SE | H | G | Hexp | E.5 | Ia | p.Ia | rbarD | p.rD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gotland | 73 | 55 | 9.5 | 0.685 | 3.87 | 38.9 | 0.988 | 0.81 | 4.62 | 0.01 | 0.419 | 0.01 |
| Kalmar | 53 | 46 | 9.54 | 0.686 | 3.72 | 32.3 | 0.988 | 0.78 | 1.69 | 0.01 | 0.147 | 0.01 |
| Skåne | 54 | 42 | 9.05 | 0.951 | 3.51 | 21.4 | 0.971 | 0.63 | 2.23 | 0.01 | 0.207 | 0.01 |
| Uppsala | 26 | 17 | 7.64 | 1.152 | 2.51 | 7.86 | 0.908 | 0.61 | 2.6 | 0.01 | 0.257 | 0.01 |
| V-götaland | 19 | 15 | 8.6 | 0.902 | 2.58 | 10.9 | 0.959 | 0.82 | 2.01 | 0.01 | 0.199 | 0.01 |
| Ö-götaland | 41 | 24 | 8.27 | 1.07 | 2.92 | 14.4 | 0.954 | 0.76 | 2.07 | 0.01 | 0.253 | 0.01 |
| Latvia | 3 | 2 | 2 | 0 | 0.64 | 1.8 | 0.667 | 0.9 | NaN | NA | NaN | NA |
| Estonia | 5 | 5 | 5 | 0 | 1.61 | 5 | 1 | 1 | 1.22 | 0.01 | 0.15 | 0.01 |
| Norway | 6 | 6 | 6 | 0 | 1.79 | 6 | 1 | 1 | 2.21 | 0.01 | 0.278 | 0.01 |
| Finland | 4 | 4 | 4 | 0 | 1.39 | 4 | 1 | 1 | 0 | 0.475 | NaN | NA |
| Total | 284 | 199 | 9.69 | 0.557 | 5.02 | 92.3 | 0.993 | 0.61 | 2.7 | 0.01 | 0.225 | 0.01 |

Table 2. Poppr analysis after elimination of loci (more than 50% missing) and individuals (more than 50% missing).

The number of multilocus genotypes (MLG) in each of the populations is high, much higher than the expected number (eMLG). The two measures of multilocus genotype diversity (H, the Shannon-Wiener index, and G, the Stoddard and Taylor index) are high, but the evenness, E.5, is low.

| | | | | | |
|---|---|---|---|---|---|
| Skåne | 42 | 1.778 | 0.0099 | 0.167 | 0.0099 |
| Uppsala | 17 | 2.595 | 0.0099 | 0.245 | 0.0099 |
| V-götaland | 15 | 1.546 | 0.0099 | 0.155 | 0.0099 |
| Ö-götaland | 24 | 1.568 | 0.0099 | 0.197 | 0.0099 |
| Latvia | 2 | NA | NA | NA | NA |
| Estonia | 5 | 1.215 | 0.0198 | 0.15 | 0.0099 |
| Norway | 6 | 2.215 | 0.0099 | 0.278 | 0.0099 |
| Finland | 4 | -0.706 | 0.8911 | -0.707 | 1 |
| Total | 216 | 2.366 | 0.0099 | 0.196 | 0.0099 |

Table 3. Index of Association (Ia) and its standardized value rbarD, with probabilities for the samples taken 2012 (after clone correction).

Redundant multilocus genotypes were then removed from the data set for calculation of index of association (Table 3). For those samples from Sweden, there was statistically significant

association of the different loci, based on the index of association. It was not significant for the samples from outside of Sweden, though there were, in general, only a few samples from each of the populations.

Minimum spanning networks were also calculated with this material. In these figures, the size of each of the circles is related to the number of individuals, and the thickness of the bars connecting them are related to how closely these individuals are related. Networks were visualized with stratification both by region (Figure 1) and by crop (Figure2).

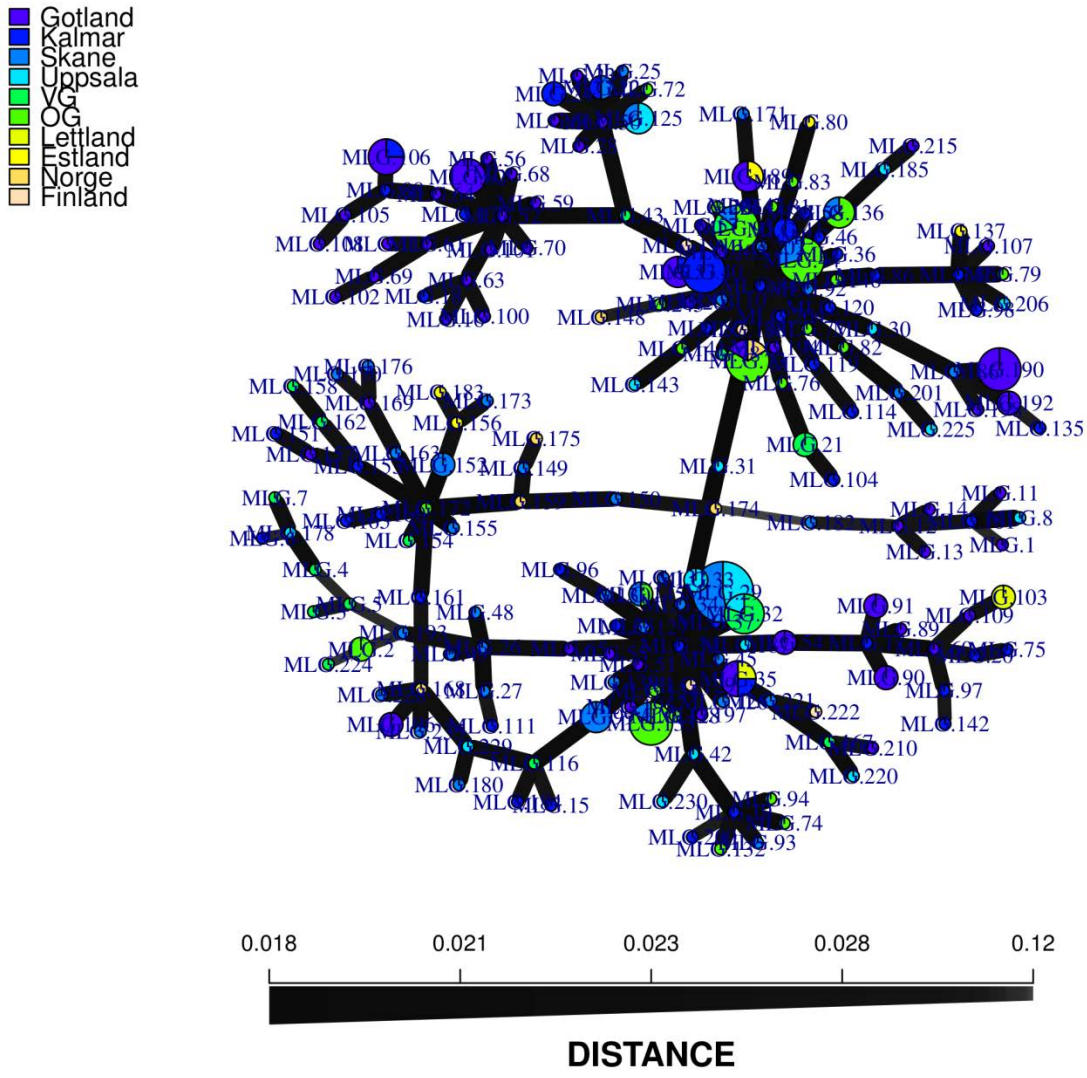


Figure 1. Minimum spanning network of samples from 2012, grouped by region

The aecia collected in 2012 were analysed by sequencing their ITS region. The samples mostly belong to the species *Puccinia graminis* and some from Öland to *P. arrhenatheri*. *P. striiformis* was not found.

The program structure was also used on a clone-corrected data set to see how the samples could be grouped. In this case, the optimum number of groups was 6 (Figure 3), and clear similarities and relationships could be seen among the different samples.

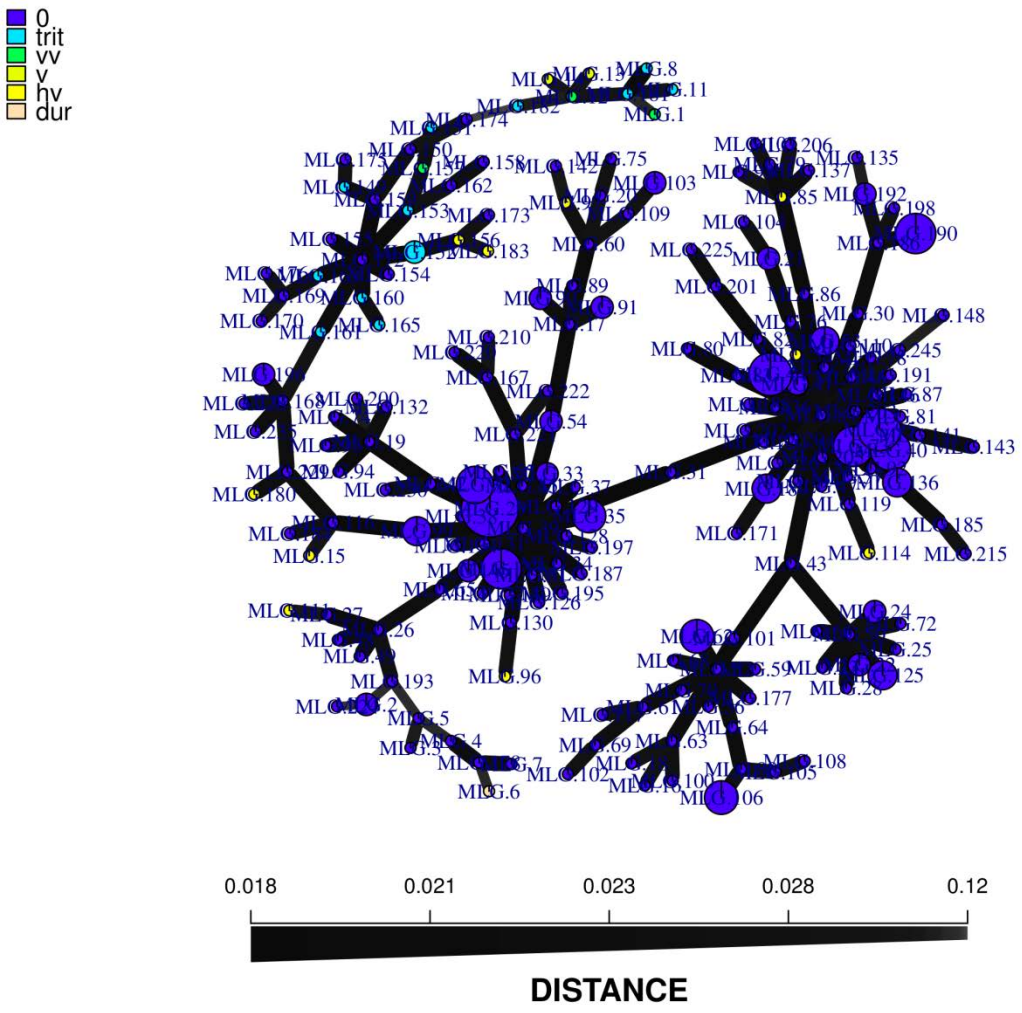


Figure 2. Minimum spanning network for the samples from 2012, grouped by crop (0 not-noted, probably winter wheat, trit: triticale, vv: spring wheat, v: unspecificied wheat, hv: winter wheat, dur: durum wheat).

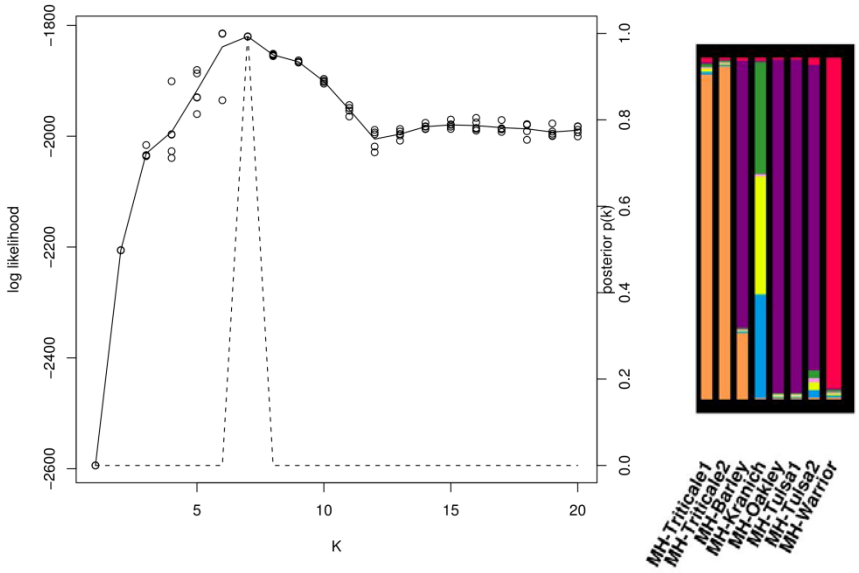


Figure 3, Log likelihood as a function of the number of groups, K. Maximum likelihood obtained when k = 7 and membership of known races from 2012.

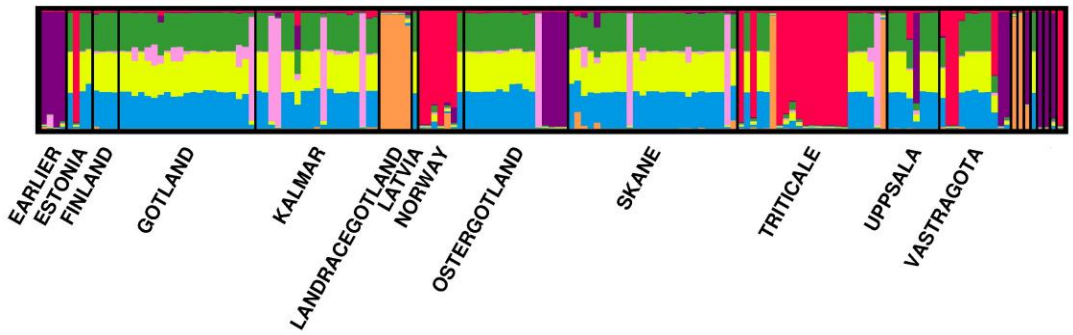


Figure 4. Structure grouping of the 2012 collection by region.

Samples from 2015 were analysed for phenotype, and the races 'Kranich', 'Warrior', a variant of 'Warrior' without virulence to Ambition, and two Triticale races were found (Table 4). Genetic analyses with poppr indicated a large number of genotypes (Table 5) and statistically significant indices of association, and these indices remained significant even after removal of clones from each field and region (Table 6). Minimum spanning networks of these samples showed similar genetic groupings as the material from 2012, whether grouped by collection area or by host crop.

| Race Name | Susceptible Yr Genes |
|---|---|
| Kranich | 1, 2, 3, 6, 7, 8, 9, 17, 25, 32 |
| Warrior | 1, 2, 3, 4, 6, 7, 9, 17, 25, 32, Sp, Amb |
| Warrior (-) | 1, 2, 3, 4, 6, 7, 9, 17, 25, 32, Sp |
| Triticale 2006 | 2, 6, 7, 8, 10 |
| Triticale 2015 | 2, 6, 7, 8, 9 |
| Hereford | 2, 3, 6, 7, 8, 25, 32 |

Table 4. Races of *P. striiformis* found in 2015.

| Pop | N | MLG | eMLG | SE | H | G | lambda | E.5 | Hexp | Ia | p.Ia | rbarD | p.rD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| hv_upp | 61 | 32 | 7.26 | 1.34 | 2.83 | 7.4 | 0.864 | 0.398 | 0.253 | 3.91 | 0.01 | 0.41 | 0.0099 |
| hv_kla | 70 | 23 | 5.72 | 1.35 | 2.19 | 4.1 | 0.756 | 0.391 | 0.234 | 3.91 | 0.01 | 0.43 | 0.0099 |
| vv_upp | 15 | 14 | 9.57 | 0.5 | 2.62 | 13 | 0.924 | 0.965 | 0.374 | 2.09 | 0.01 | 0.18 | 0.0099 |
| trit_ryd | 14 | 11 | 8.52 | 0.74 | 2.34 | 9.8 | 0.898 | 0.936 | 0.452 | 4.9 | 0.01 | 0.49 | 0.0099 |
| hv_ola | 2 | 1 | 1 | 0 | 0 | 1 | 0 | NaN | 0.4 | NA | NA | NA | NA |

Table 5. Summary of genetic information from 2015 (hw: winter wheat, vv: spring wheat, trit: triticale, upp: Uppåkra, kla: Klagtorp, ryd: Rydsgård, ola: Öland).

| Pop | N | Ia | p.Ia | rbarD | p.rD |
|---|---|---|---|---|---|
| hv_upp | 32 | 3.01 | 0.01 | 0.313 | 0.01 |
| hv_kla | 23 | 3.12 | 0.01 | 0.354 | 0.01 |
| vv_upp | 14 | 2.03 | 0.01 | 0.174 | 0.01 |
| trit_ryd | 11 | 3.66 | 0.01 | 0.374 | 0.01 |
| hv_ola | 1 | NA | NA | NA | |
| Total | 69 | 2.86 | 0.01 | 0.232 | 0.01 |

Table 6, Index of Association (Ia) and its standardized value rbarD, with probabilities, from the samples taken in 2015 after clone-correction. See table 5 for abbreviations.

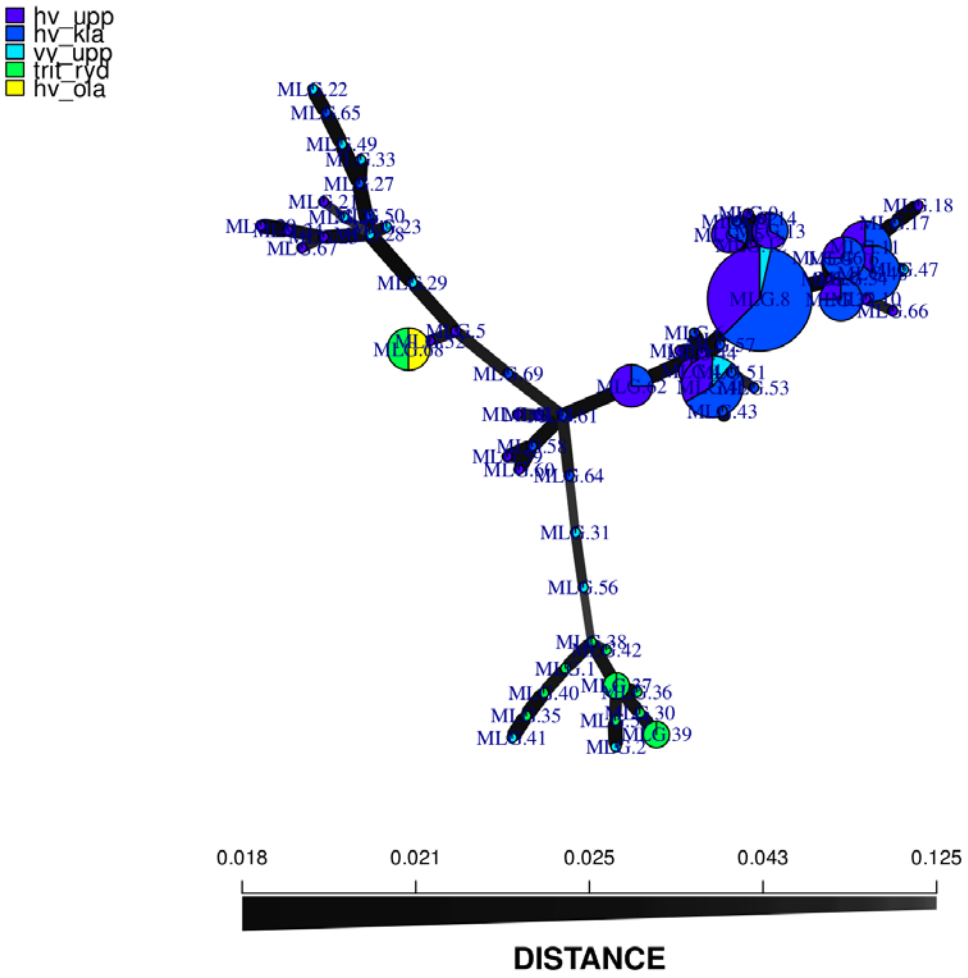


Figure 5. Minimum spanning network from 2015 samples, grouped by region/field trial. 2015 (hw: winter wheat, vv: spring wheat, trit: triticale, upp: Uppåkra, kla: Klagtorp, ryd: Rydsgård, ola: Öland).

## Discussion

There were a number of staffing difficulties encountered in this project. The most serious was that the PhD student originally recruited had decided not to continue her studies. The remainder of the work was done by other staff at SLU and a master student, since there were insufficient funds left to finance a new PhD student.

Though the *P. striiformis* population exhibits variation when examined with the microsatellite markers, there are a number of clones present in the material, and identical (or near identical) individuals have spread to different fields. There is clear grouping of genetically similar, but not identical, individuals when minimum spanning networks are constructed using the Bruvo distance. This, together with the significant index of association, shows that the genetic structure of *P. striiformis* shows none of the characteristics one would expect for a sexually reproducing population. An added piece of evidence is that no aecia of *P. striiformis* were recovered from barberry. Thus, there cannot be large amounts of sexual reproduction taking place in Sweden.

Sexual reproduction may be possible to a limited extent, but the effects would be masked by the dominating clonal lineages. The progeny from sexual events would need to be better competitors than the rest of the population, and would not be evident till they had reached large numbers. At

that point, they would manifest themselves as another clonal lineage, and one would not be able to determine if the sexual event took place in Sweden or in another country, from whence the genotype had emigrated.

There does not appear to be any geographical grouping of the clonal lineages, and there was no clear geographical pattern observed in the minimum spanning networks. The analyses with structure revealed some geographical similarities, with similarities between the Norwegian material and that found on triticale. The material from the older landraces on Gotland were also unique.

Structure also indicated some inconsistent relationships in the analysis of some of the known races, and these analyses should be repeated, since contamination cannot be ruled out.

The genetic analyses were able to group the lineages based on host crop to a limited extent. While the lineages on wheat seem to form several groups, the ones on triticale seem to be somewhat separate. One exception seems to be the material from Öland in 2015, where genetically identical individuals were found on both triticale and winter wheat.

The races of *P. striiformis* that were found in 2015 are similar to those found in other European studies. Of particular note is that the Warrior/Ambition race was recovered in two variants, as was the Triticale race.

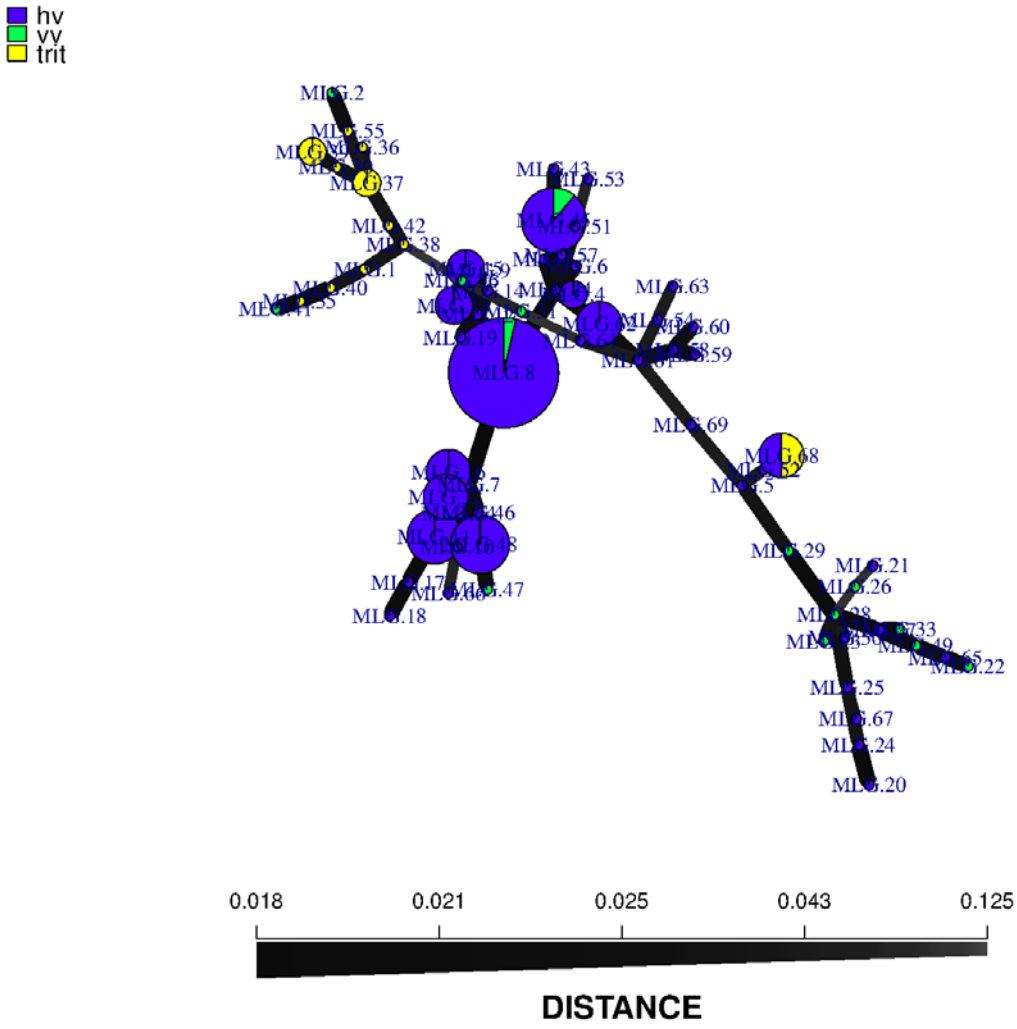


Figure 6. Minimum spanning network from 2015 samples, grouped by crop.

## Publications

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Nilsson, M. 2016. Populationsstudie av gulrost (*Puccinia striiformis*) I Skåne under odlingssäsongen 2015. Magisterarbete 30 hp, Institutionen för skoglig mykologi och växtpatologi, SLU, Uppsala.

Wan, A.M, Chen, X.M., and Yuen, J. 2016. Races of *Puccinia striiformis* f.sp. *tritici* in the United States in 2011 and 2012 and comparison with races in 2010. Plant Diseae *in press*.

## Conclusions

Sexual reproduction of *P. striiformis* is either very rare or does not occur in Sweden. Substantial variation in the pathogen exists, which indicates that it is able to adapt to different conditions despite a primarily clonal existence. Molecular markers can be useful for identifying clonal lineages but their usefulness in being able to identify races depends on how easily new races can be produced by the pathogen. If the pathogen is sufficiently variable, such a strategy would work for only a limited amount of time.

## Take Home Message to Agricultural Producers

Barberry eradication is probably not needed in order to reduce the impact of stripe rust under the current Swedish conditions, though it still contributes to problems with stem rust on oats. The stripe rust pathogen is very variable, and seems to be able to develop new races without any obvious sexual reproduction. If the use of resistant varieties is to be pursued as a disease management strategy, monitoring of races is of prime importance, as well as additional knowledge as to how and how often these new races arise. Expression studies in wheat plants may provide a better molecular method for race identification since the markers used in this study could identify genetic relationships but race determination is still dependent on greenhouse tests with inoculated plants.

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