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Digging deeper into the evolutionary history of *Rhynchosporium secalis*

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A mi esposo y a los hijos que tendremos,
ellos son mi fortaleza día tras día.

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Abstract

24 field populations sampled in Europe and Ethiopia were assayed with eight microsatellite loci. Model based methods were used to infer the population structure and recent migration patterns. Allelic richness obtained in our study is consistent with the hypothesis of Scandinavia as center of origin. A population structure consisting in two different levels, namely population and region, was proposed. The AMOVA analysis showed that 80% of the variation was explained by the population level and 6% by the regional level. Isolation by distance was confirmed by Mantel test analysis. However, Icelandic, Ethiopian and Swiss populations did not fit such a model. The dynamic of migration of the Icelandic populations was consistent with a continent island model closer to the coalescence point. According with the geographic characteristics of Iceland in relation with the proposed center of origin, an active movement of infected seed mediated by humans is proposed to explain our results. In contrast, Swiss populations located in the same continent with the proposed center of origin, showed the highest differentiation values. Based on a recent migration analysis, we proposed that *R. secalis* populations are being modeled by human mediated migration.

Resumen

24 poblaciones de *R. secalis* muestreadas en Europa y África fueron evaluadas para ocho loci microsatellites. Métodos basados en modelación fueron usados para inferir la estructura de la población y los patrones recientes de migración. Los índices de riqueza alélica obtenidos en nuestro estudio son consistentes con la hipótesis de Escandinavia como centro de origen. Una estructura poblacional de dos niveles, población y región, fue usada en nuestro estudio. El análisis de AMOVA mostro que el 80% de la varianza puede ser explicada por el nivel población y que un 6 % es explicado por el nivel región. El patrón de aislamiento por distancia fue confirmado mediante el test de mantel, a pesar de que las poblaciones de Etiopía, Islandia y Suiza no se ajustaron a dicho modelo. La dinámica de migración de las poblaciones de Islandia correspondió con un modelo isla continente cercano al punto de coalescencia. De acuerdo con la localización geográfica de Etiopía, en relación al centro de origen propuesto, un movimiento activo de semilla contaminada mediado por humanos es propuesto para explicar nuestros resultados. En contraste, los poblaciones Suizas, que se localizaron en la misma área continental que el propuesto centro de origen mostraron los valores más altos de diferenciación, De ésta forma se propone que las poblaciones de *R. secalis* están siendo modeladas por la migración mediada por humanos.

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Introduction

Rhynchosporium secalis (Oudem.) J.J. Davis is the causal agent of the worldwide disease, barley scald. The global distribution and economic importance of this pathogen have motivated several studies focused on its biology and population dynamics (Zhan *et al*., 2008). This pathogen is a haploid organism whose teleomorph has never been identified (Salamati *et al.,* 2000; Zaffarano *et al.,* 2006). For organisms with an unknown sexual stage, recombination can only be inferred by indirect methods (Milgroom, 1996; Taylor et al., 1999). In the case of *R. secalis,* a cryptic sexual stage has been proposed based on indirect evidences (McDermott, *et al.,* 1989; Goodwin *et al.,* 1993; McDonald *et al.,* 1999; Salamati *et al.,* 2000; Linde *et al.,* 2003; Zaffarano *et al.,* 2006).

It has been proposed that some plant pathogens were domesticated along with their host (Stukenbrock and McDonald, 2008). Current information suggests that *R. secalis* did not follow such an evolutionary pattern (Zaffarano *et al.,* 2006, 2008 and 2009; Linde *et al.,* 2009). In agriculture, the center of origin is considered as the area where an organism was domesticated and frequently is also the area with highest genetic diversity (Stukenbrock and McDonald, 2008). Based on genetic and archeological evidence the center of origin of barley (*Hordeum vulgare* L.) is located within the Fertile Crescent, in the Middle East (Badr *et al.,* 2000). In the case of *R. secalis*, populations with the highest genetic diversity are from Scandinavia. Hence, Northern Europe has been proposed as the center of origin of *R. secalis* (Zaffarano *et al.,* 2006, 2008 and 2009; Linde *et al.,* 2009). Based on previous data, it has been hypothesized that barley crops were introduced from the Middle East to northern Europe, where they came into contact with the ancestor of *R. secalis* populations (Zaffarano *et al.,* 2008). Then, *R. secalis* emerged as a disease through colonization of domesticated barley, following the host shift model (Zaffarano et al., 2008).

Modern plant pathogen populations are shaped by the evolutionary forces that have acted on their ancestral populations (Barrett *et al.,* 2008; McDonald and Linde, 2002). Gene flow, one of these evolutionary forces, is defined as the modification in population allele frequencies caused by effective migration events (Slarkin, 1985). In order to understand gene flow and migration patterns, population boundaries must be defined. In the case of plant pathogens, population boundaries are not easily identified, therefore, a study of migration requires an approach relying on the structure of the population instead of predefined populations. Inaccurate definition of the population structure may produce distorted results in terms of the amount of the genetic variation and values of gene flow among populations.

Recently, several model-based methods to infer population structure have been successfully introduced (Pritchard *et al* .2000; Dawson & Belkhir 2001; Falush *et al*. 2003; Corander *et al.,* 2006; Corander *et al.,* 2008). These model based methods are able to infer the correct number of clusters even in scenarios with relatively low level of genetic differentiation among populations (Latch *et al*. 2006). Therefore, model based methods represent an accurate approach to clarify the population structure based exclusively on genetic data.

Dispersion strategies determine the migration patterns of an organism (Slarkin, 1985). Zaffarano et al. (2006) concluded for global populations of *R. secalis* that there is significant gene flow on a regional scale, and more restricted gene flow between regions. Conidia produced by the asexual stage are splash-dispersed, therefore, responsible for local or within field movement (Zaffarano et al. 2006; Fountaine *et al.*, 2010). Zaffarano et al. (2006) also suggested that wind-dispersed *R. secalis* ascospores, from the unknown sexual stage, could explain the high degree of similarity on a regional scale. Hence, the natural dispersion of the putative ascospores could use a stepping-stone model and through a movement of only a few kilometers at a time, link together barley fields over large areas within barley-growing regions. Another hypothesis proposed that *R. secalis* inoculum could be carried on infected seeds (Habgood, 1971; Jackson and Webster, 1976; Fountaine *et al.*, 2010). Supporting this idea, Linde et al. (2009) reported molecular evidence suggesting human mediated migration for the barley scald pathogen. In order to test these emerging hypotheses, a detailed study needs to be conducted.

The main objective of this study was to infer the population structure and recent migration patterns in European and African populations of *R. secalis*. We analyzed populations from Finland, Norway, Iceland, Switzerland, Spain, Jordan, Syria and Ethiopia. We addressed the following questions: Is there a hidden structure in the populations of our study? How the pattern of isolation by distance in *R. secalis* has been influenced by geographical barriers in continental and islander locations? How the current population structure of *R. secalis* is shaped by the pathogen dispersion strategies or human mediated migration? Our null hypothesis was that *R. secalis* recent migration patterns could be explained exclusively by its own means of biological dispersion instead of human mediated migration.

1. Materials and Methods

1.1 R. secalis populations

1072 *R. secalis* isolates were included in this study, representing 24 field populations, each originating from a single barley field. 18 of these field populations were described in previews studies and were sampled using transects or hierarchical strategies. The additional six populations were sampled in Iceland and Spain following a hierarchical strategy. Table 1 summarizes the location details for each population. For the fungal isolation and culturing we followed the methodologies described previously by McDonald *et al*. (1999). The DNA was extracted with the DNeasy Plant Mini DNA extraction kit (Qiagen GmbH, Germany) according to the specifications of the manufacturer.

1.2 Microsatellite genotyping

All individuals were genotyped at eight microsatellite loci: RH1, RH2, RH4, RH5, RH6, RH8*, RH11 and RH14 (Linde et al., 2005). Linde et al., (2009) found no evidence of selection acting on these loci, thus they were assumed to behave as neutral markers. PCR was performed with the fluorescently labeled primers and conditions described previously by Linde et al. (2005), but every pair of primers was used in independent reactions. Allele sizes were recorded by comparison to fluorescently labeled size standard (GeneScan-500 LIZ; Applied Biosystems) in an ABI PRISM 3100 automated sequencer (Applied Biosystems). GeneMapper Software v4.1 (Applied Biosystems) was used to score the size of the PCR products.

Table 1: Description of *R. secalis* populations

Predefined region	Country	Field population[a]	Location Latitude	Longitude	Source and year of collection or previous publication
Middle East	Syria	SYR A (1)	36°37´	37°51´	Zaffarano *et al*. (2006)
		SYR B (2)	36°37´	37°51´	Zaffarano *et al.* (2006)
		SYR C (3)	36°33´	38°48´	Zaffarano *et al.* (2006)
		SYR D (4)	36°33´	38°48´	Zaffarano *et al*. (2006)
		SYR E (6)	33°14´	35°45´	Zaffarano *et al*. (2006)
	Jordan	JOR A (1)	29°59´	35°83´	Zaffarano *et al*. (2006)
		JOR B (2)	29°59´	35°83´	Zaffarano *et al*. (2006)
Switzeland	Switzerland	CH1 (CH99.2-Luegern)	47°58´	8°21´	Linde *et al*. (2003)
		CH2 (CH99.5-Lucens)	46°70´	6°83´	Linde *et al*. (2003)
		CH3 (CH99.6-Cugy)	46°58´	6°64´	Linde *et al*. (2003)
		CH4 (00CH-Zürich)	47°36´	8°53´	Linde *et al.* (2003)
		CH5 (01CH1-Eschikon)	47°44´	8°67´	Linde *et al*. (2003)
		CH6 (01CH2-Dietikon)	47°40´	8°40´	Linde *et al.* (2003)
Scandinavia	Norway	CNor (Central Norway)	66°74´	14°58´	Salamati *et al*. (2000)
		SENor (Southern Norway)	59°38´	11°07´	Salamati *et al*. (2000)
	Finland	FIN	61°92´	25°74´	Salamati *et al.* (2000)
Iceland	Iceland	IceH	65°41´	19°22´	Stefánsson (2008)[b]
		IceG	63°59´	20°56´	Stefánsson (2008)[b]
		IceK	64°09´	21°44´	Stefánsson (2008)[b]
Spain	Spain	SpA	41°50´	-5°74´	F. Garcia-Arenal (2005)[b]
		SpB	40°03´	-3°60´	F. Garcia-Arenal (2005)[b]
Ethiopia	Ethiopia	ET03A	11°34´	37°97´	Linde *et al* (2009)
		ET03T	14°03´	38°31´	Linde *et al* (2009)
		ET03O	7°54´	40°63´	Linde *et al* (2009)

a Population names in parentheses refer to names used in previous publications
b Collaborators who kindly provided diseased plant material.

1.3 Data analyses

1.3.1 Haplotipic diversity

The number of private alleles and allele frequencies were estimated using the program CONVERT version 1.31 (Glaubitz J.C., 2004). Isolates with the same haplotype were treated as clones. For each clone, only one representative was chosen to construct a clone-corrected data

set. Determination of haplotypes and clone correction was performed using the program GENODIVE (Meirmans and Van Tienderen, 2004). Indices of clonal diversity as i) the number of haplotypes per population and global; ii) the clonal fraction calculated as 1 – [(number of different genotypes)/(total number of isolates)]; and iii) the Stoddart and Taylor's genotypic diversity $Go = 1/\Sigma pi^2$, where pi is the frequency of the ith genotype; were also calculated using GENODIVE.

1.3.2 Gene diversity

Genetic variation was quantified by measures of allelic richness and gene diversity. Nei's unbiased gene diversity and comparisons of those values among the populations were estimated using the program FSTAT 2.9.3 (Goudet, 2001). Allelic richness was calculated as the mean number of alleles per locus using rarefaction (Mousadik and Petit, 1996; Petit et al., 1998). Nei's unbiased gene diversity, corrected for sample size, was calculated according to Nei (1978) as $n/(n-1) \times (1 - \Sigma_i p_i^2)$, where p is the observed frequency of the ith allele and n is the sample size. To compare whether populations differed for allelic richness and Nei's gene diversity, a bootstrapping approach based on 1,000 permutations was performed using FSTAT 2.9.3.

1.3.3 Population structure

Two different options of the Bayesian clustering algorithm implemented in BAPS 5.1 (Corander *et al.,* 2008; Corander *et al.,* 2006) were used to identify a possible cryptic population structure. BAPS 5.1 (Corander *et al.,* 2008; Corander *et al.,* 2006) relies on stochastic optimization to infer the posterior mode of genetic structure. In this study, a first approach without any prior geographic information was used to identify the optimal number *K* of partitions among groups of samples. The group-level option in BAPS 5.1 (Corander *et al.,* 2008; Corander *et al.,* 2006) was used such that clusters are formed by assembling whole samples. The program was run for *K* ranging from 1 to 25 with five replicates for each value of *K* to ensure that the stochastic optimization algorithm had not ended up in different solutions in separate runs. According to the results, a second approach was used to evaluate if an additional level in the structure which grouped different populations, namely region, could be associated with the data. Different fixed values of K ranging from 12 to 1 were tested. Goodness-of-fit of the clustering solutions to the dataset were compared in terms of the marginal likelihood of the data.

1.3.4 Differentiation among populations

The differentiation among geographical populations was assessed based on G_{ST} pairwise comparisons of populations and the analysis of molecular variance (AMOVA) using GENALEX 6.0 (Peakall and Smouse, 2006). The Gst (analog to Fst in diploid organisms) was used as the distance measure to calculate those statistics instead of Rst because Rst assumes a strict step-wise mutation model and does not consistently outperform Fst measures (reviewed in Pearse and Crandall, 2004).

1.3.5 Isolation by distance

Mantel tests were performed using the procedure of Smouse *et al*. (1986), which estimates matrix correspondence using a measure (*Rxy*) analogous to an autocorrelation coefficient. The procedure is implemented within the program genalex 6.0 (Peakall & Smouse, 2006). The statistical significance of the values was assessed via 999 random permutations.

1.3.6 Recent migration rates

Based on the results of the structure analysis, we estimated the recent migration events using the Bayesian admixture analysis (with the predefined populations option) in BAPS 5.1 (Corander *et al.,* 2008; Corander & Marttinen*,* 2006). This program estimates the optimal posterior mode of the proportion of an individual's multilocus genotype that is represented by each population. For this analysis, we used 1000 interactions to estimate the admixture coefficients for the individuals, we used 200 reference individuals from each cluster, and we repeated the admixture analysis 50 times per individual.

2. Results

2.1 Haplotypic diversity

From an initial number of 1072 individuals, we identified 576 haplotypes using 8 microsatellite loci, representing 195 alleles in total (Table2). All the loci were polymorphic in all populations. The number of alleles per locus ranged from 9 in RS1 to 36 in RS14 with an average of 24.3. Alleles frequencies varied depending on the loci. While in five loci any allele showed a frequency higher than 24%, only three loci had frequencies distributed unevenly. The most frequent allele for the loci RS1, RS4 and RS11 displayed values of 34%, 53% and 38% respectively.

Table 2: Genetic diversity indexes for *R. secalis* field populations grouped in six predefined regions.

Region	Sample size (N)	Number of haplotypes	Number of alleles	Number of private alleles	Clonal fraction	Genotypic diversity (Go)	Allelic richness		Gene diversity (Nei, 1987)	
Scandinavia	180	107	117	12	40.56	64.8	13.28	b	0.783	ab
Iceland	114	90	90	8	21.05	78.289	10.46	a	0.725	a
Switzerland	200	107	79	14	46.50	42.283	8.94	a	0.57	a
Spain	132	60	82	4	54.55	37.231	10.18	a	0.780	a
Middle East	349	105	90	12	69.91	16.706	10.05	a	0.67	a
Etiopia	135	107	82	11	20.74	95.41	9.7	a	0.768	a
TOTAL	1072	576	195							

Go. Stoddart and Taylor's genotypic diversity $Go = 1/\Sigma pi^2$, where *pi* is the frequency of the *i*th genotype (Meirmans and Van Tienderen, 2004).

2.2 Gene diversity

Field populations were pooled to represent 6 predefined regional groups based on geographic location: Iceland, Scandinavia, Switzerland, Spain Middle East and Ethiopia (Table1). The variation among the populations within these regions ranged from 0% in Spain to 8.7% in Ethiopia (Table 3). The clonal fraction was moderate in most of the regions. Ethiopia (20.7) and Iceland (21.05) had the lowest values while Middle East (69.9) showed the highest one. Consequently, in terms of genotypic diversity, Ethiopia and Iceland had the highest values while Middle East had the lowest one. After rarefaction to 60 individuals, the values of allelic richness were distributed between 8.9 (Switzerland) and 13.28 (Scandinavia). Gene diversities were moderate and similar among all regional populations with values ranging from 0.57 in Switzerland to 0.78 in Spain and Scandinavia. Bootstrap analysis revealed that values of allelic richness were significantly different between Scandinavia and the rest of the regions. (P,0.001). The amount of private alleles is a measure sensitive to sample size. Among the groups with similar sample sizes (Scandinavia, Switzerland, Middle East and Ethiopia), the highest number of private alleles was observed in the populations from Switzerland (14), followed by Middle East and Scandinavia, both with the same value (Table 2). Even though the sample size of Iceland was smaller, 8 private alleles were detected.

Table 3: Analysis of molecular variance of four *R. secalis* structure designs. Designs i and ii correspond to predefined structure according to sampling scheme. Designs iii and iv were suggested by BAPS clustering analysis. Northern Europe, which encompasses Scandinavia and Iceland, is treated as one region in the designs iii and iv but in the analysis I and ii, Iceland is treated as a different region.

			Percentage of variation		
Sample	Number of Regions	Numbers of populations	Among Regions	Among populations within Regions	Within populations
i. Field populations	1	24		20	80
ii. Predefined geographic regions	6	24	17	5	78
Scandinavia	1	3		3.9	96.0
Iceland	1	3		6.9	93.0
Switzerland	1	6		4.7	95.2
Spain	1	2		0.0	100
Middle East	1	7		7.4	92.5
Ethiopia	1	3		8.7	91,2
iii. BAPS suggested clusters	1	8		20	80
iv. Two levels BAPS design	5	8	15	5	80

2.3 Population structure

The posterior probabilities obtained by the BAPS 5.1 analysis, without any prior information, supported an optimal K number of 8 inferred populations out of 24 field populations: 1) SYR A+B+C+D+E + JOR A+B, 2) CH 1+2+3+4+5, 3) CH 6, 4) CNor-+SENor+IceH+FIN, 5) SpA+B 6) IceG+K, 7) ET03A+03O, 8) ET03T. The inferred population CNor+SENor+IceH+FIN consisted of one field population from Iceland, one from Finland and two field populations from Norway (Figure 1).

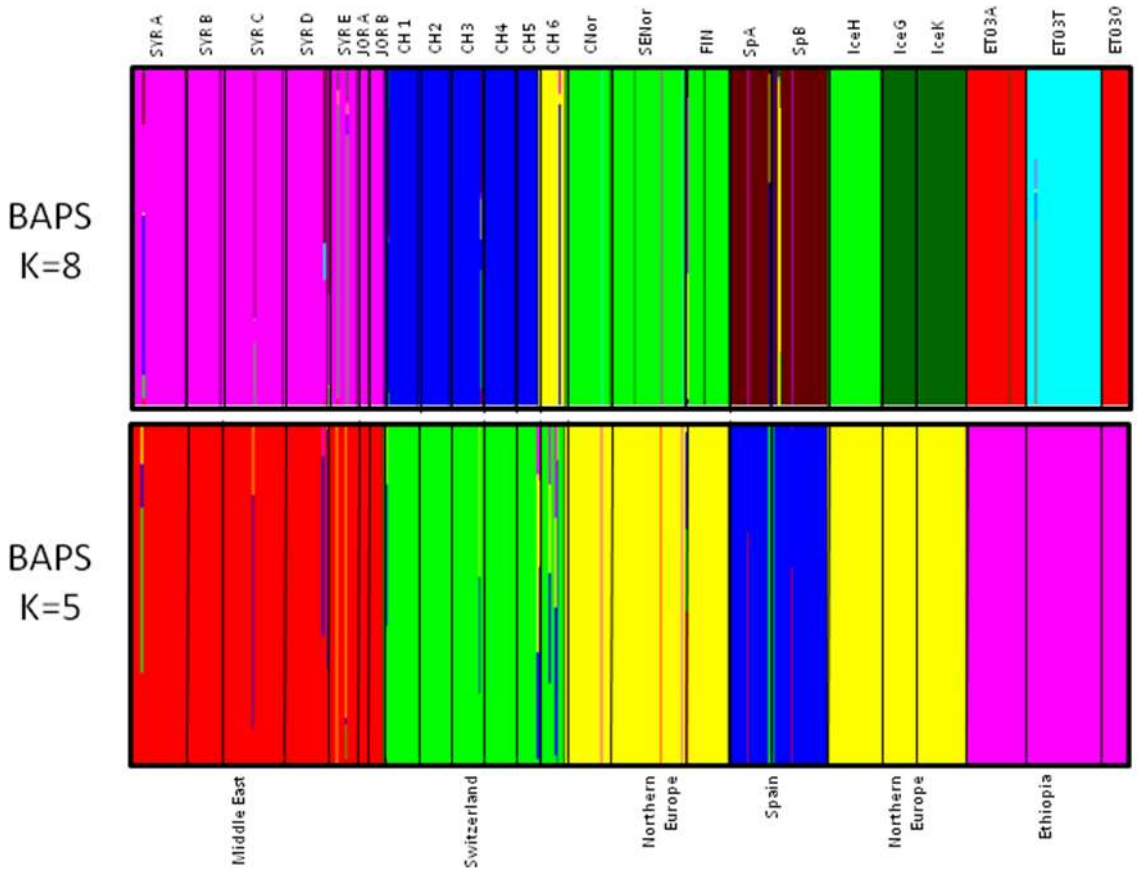

Figure 1 : Estimated population structure. A vertical line partitioned into K colored segments represents the individual´s estimated membership in K clusters. Black lines separate individuals of different populations. Population labels are showed above the figure and regional affiliations below it. The figure at K=8 corresponds to the result when BAPS 5.1 was run for *K* ranging from 1 to 25 with five replicates for each value of *K* to ensure that the stochastic optimization algorithm had not ended up in different solutions in separate runs. The figure at the bottom shows a second approach with K values ranging from 8 to 1, which allow us to detect a second level in the structure, namely region. The figure shown for a given K is based on the highest probability run at that K.

In the analysis made using fixed values of K, from 8 to 1, each decrease in K merged two of the clusters obtained with the previous K value. At K=5, the formed regions corresponded largely to the geographic distribution of the samples. The inferred regions

consisted of populations from Ethiopia, Middle East, Spain, Switzerland and Northern Europe. This analysis also revealed a close relationship between Icelandic and Scandinavian populations. Therefore, those predefined geographic regions were merged to form one single region, Northern Europe (Figure 1). Finally, based on these results, we proposed a two levels design of population structure; regions (5) and groups of field populations (8).

2.4 Differentiation among populations

The analysis of molecular variance (AMOVA) was performed to describe the distribution of the genetic variation at different levels for each one of the structures suggested previously: i) First, based on the field populations; ii) predefined structure based on geographic regions; iii) structure suggested by BAPS 5.1 forming 8 inferred populations; and iv) two level structure design suggested by BAPS 5.1 with inferred regions (5) and inferred populations (8).

Most of the variation was distributed within populations (78%-80%) for all of the different designs of population structure. In the designs of one hierarchical level (i and iii) the variation within populations was 80%. In the designs with two levels (ii and iv), populations and regions, the variation among populations showed by the one cluster designs, was partitioned into variation among regions (17% and 15% respectively) and variation among populations within regions (5%).

In order to know if the AMOVA analysis supported the two levels design suggested by BAPS, the differentiation within and among predefined regions was calculated. The differentiation values within each region ranged between 0% and 8%, (Table 3) suggesting no substructure within those regions. The pairwise comparisons of the differentiation among the predefined regions showed Gst values between 0.063 and 0.353 (table 4). The lowest value was found between Scandinavia and Iceland. This value supports the results of BAPS 5.1 where Iceland and Scandinavia were merged together in the same region, Northern Europe. Switzerland was the region most strongly differentiated from all other regions (Gst values ranged from 0.241 to 0.353) . As a consequence, the AMOVA analysis supported the two levels structure design (iv).

2.5 Isolation by distance

The correlation coefficient between the differentiation value (Gst) and the geographical distance among 24 field populations was 0.27 ($p<0.003$). When the Icelandic field populations were excluded from the analysis the correlation coefficient was 0.38 ($p<0.002$). These results suggest that there is isolation by distance in *R. secalis* populations of our study. However, an increase of the correlation coefficient when Icelandic populations are not included supports the idea that those populations do not fit the isolation by distance pattern.

Table 4: Population differentiation (Gst) among populations within six predefined *R. secalis* regional.

	SC	I	CH	SP	ME	ET	
SC		0.004	0.001	0.001	0.001	0.001	SC
I	0.063		0.001	0.001	0.001	0.001	I
CH	0.241	0.274		0.001	0.001	0.001	CH
SP	0.068	0.115	0.250		0.001	0.001	SP
ME	0.160	0.203	0.353	0.181		0.001	ME
ET	0.157	0.195	0.268	0.132	0.244		ET
	SC	I	CH	SP	ME	ET	

PhiPT Values below diagonal. Probability values based on 999 permutations are shown above diagonal

2.6 Recent migration rates

The admixture analysis made with BAPS 5.1 showed that 96% of the individuals were assigned to their cluster of origin with more that 95% of confidence. 3.65% of the individuals were product of ancestral admixture. Figure 2 shows a network of 8 clusters where the direction and amount of gene flow is indicated by arrows with a correspondent value. The values of gene flow indicated by the arrows should be interpreted as the proportion of the sink population explained by the makeup of the source population. For

instance, our analysis suggests that 5.6% of the migrants of CH 6 were introduced by gene flow from CH 1+ 2+ 3+ 4 + 5. The figure shows gene flow values above 0.01.

All the inferred populations showed high values associated with self looping arrows. Therefore, it is considered that most of the individuals are descendents from its own original inferred populations instead of a product of migration. The highest value of gene flow displayed by our analysis was 0.056. That value was presented by CH 1+2+3+4+5 to CH 6.

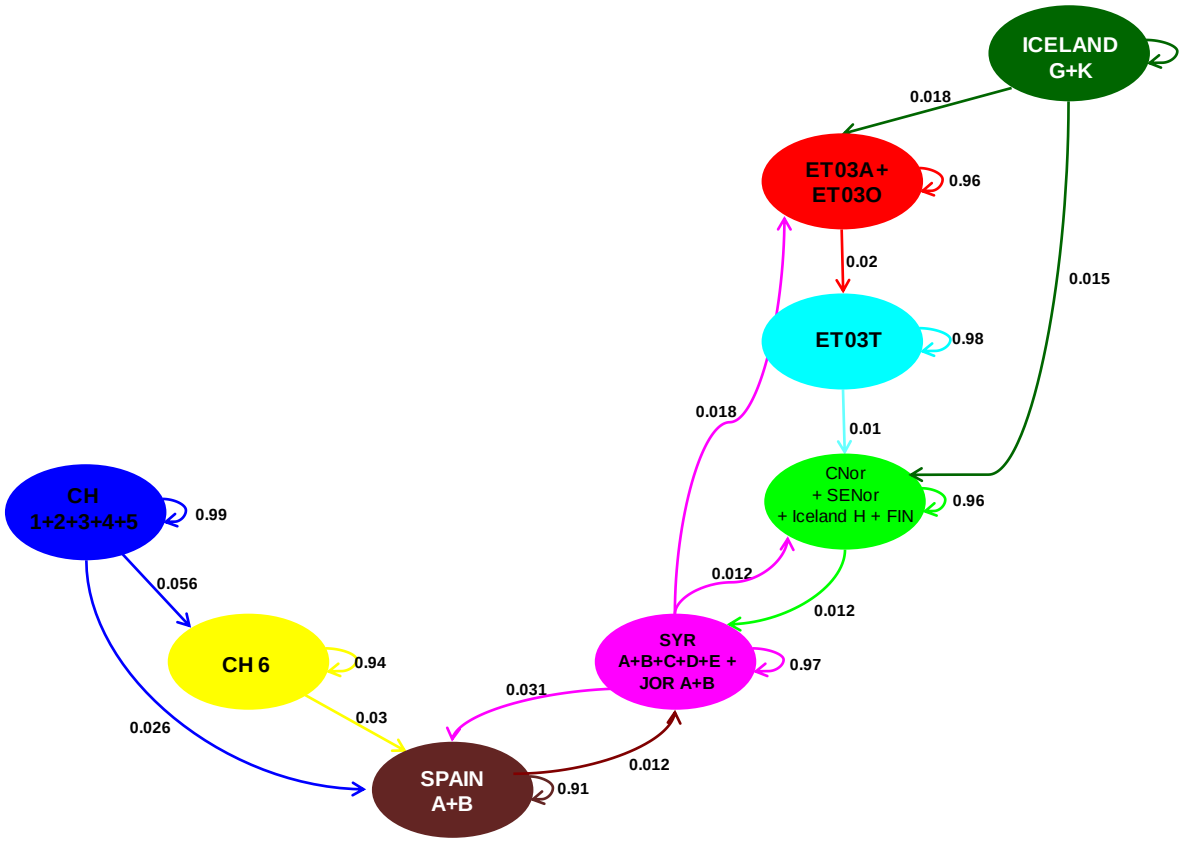

Figure 2: Gene flow network of 8 *R. secalis* inferred populations where the direction and amount of gene flow is indicated by arrows with a correspondent value. The values should be interpreted as the proportion of the sink population explained by the makeup of the source population. For instance, our analysis suggest that 6,6% of the CH6 migrants were introduced by gene flow from CH1+2+3+4+5. The figure shows gene flow values above 0.01.

The admixture analysis showed that the interchange of gene flow among the groups of populations within each of the regions is higher than among regions. This was consistent with the AMOVA values.

Figure 2 reveals a clear gene flow pattern between Northern Europe and Ethiopia. A cluster formed by two populations from Iceland (IceG and IceK) showed unidirectional flow towards Scandinavia and Ethiopia (ET03A and ET03O). The Ethiopian field population ET03T formed a separated cluster, receiving migrants from the other two Ethiopian populations and being a source of migrants for Scandinavia. Spain and Middle East were the hub of our gene flow diagram. Spain received migrants from two regions, Switzerland and Middle East, and was a donor only to Middle East. The Cluster corresponding to Middle East was a source of migrants not only for Spain but also to Ethiopia and Northern Europe. At the same time, Middle East was an acceptor of migrants from Spain and Northern Europe. Switzerland didn't receive any migrants but was connected to the diagram as source of migrants to Spain.

3. Discussion

Our null hypothesis was that *R. secalis* recent migration patterns could be explained exclusively by its own means of biological dispersion. A model based approach was used to study the population structure of *R. secalis* in Europe and Northern Africa. Detailed knowledge about *R. secalis* dispersion patterns was obtained in order to test the effect of pathogen dispersion means and human mediated transport on the current pathogen distribution.

Our analysis suggested that *R. secalis* populations could be structured in two levels. The first level, the inferred population, corresponded to a group of field populations undergoing high genetic interchange and consequently, showing low values of genetic differentiation. For example, field populations from Jordan and Syria were grouped together in one inferred population. Based on RFLP markers, Zaffarano *et al*. (2006) proposed that it is plausible to find a dynamic movement of inoculum over a spatial scale higher than 100 km. We found five out of eight inferred populations grouping field populations separated by more than 100 km, supporting the conclusion of Zaffarano *et al*. (2006) (i. Spain A+B; ii. CH 1+2+3+4+5; iii. SYR A+B+C+D+E+JOR A+B; iv. CNor+SENor+Iceland H+FIN; and v. ET03A+ ET03O showed in figure 2). The second level, the inferred region, clustered inferred populations with high similarity levels but not enough to be considered only one inferred population. Northern Europe, Switzerland, Spain, Middle East and Ethiopia were identified as inferred regions. This level represented five percent of the genetic variance based on the AMOVA analysis. Hence, the gene flow among inferred populations within a region is less dynamic than gene flow within inferred populations. However, gene flow among inferred populations within inferred regions remains as an important factor suggesting that inoculum movement within the inferred regions is more active than among inferred regions.

Northern Europe was the inferred region covering the highest geographic area (2300 km) which clustered populations from Norway, Finland and Iceland. This is the first large scale study of *R. secalis* population structure that includes field populations from Iceland. Iceland is located approximately 2300 km away from the Scandinavian Peninsula across the Norwegian Sea. It is not known if *R. secalis* was an introduced pathogen in Iceland, and it is therefore not know when it was introduced. As reviewed by Stefansson (2009), Nordic Vikings introduced barley from Norway into Iceland, soon after its settlement in 874 AD. It was grown at several locations until about 1300 AD when the price of imported barley from Scandinavia decreased significantly and cultivation of barley in Iceland essentially ceased. It was not until 1923 that barley was successfully grown in Iceland again for several years in a row. Recently, *R. secalis* has been identified as a serious pathogen of economic importance in Iceland.

If *R. secalis* was an introduced pathogen in Iceland, we would expect that its migration pattern fitted a continent-island model. In the continent-island model, gene flow is essentially unidirectional from a very large population to a smaller population. The continent population is so large that allele frequencies are not impacted by emigration or drift, whereas allele frequencies in the small population(s) are strongly influenced by immigration. After multiple and continuous migration events from mainland to island, the diversity differences between the island and continent decrease toward zero over time and the diversity of the island approaches the diversity of the mainland (Hamilton, 2009).

Diversity indexes of Icelandic populations were higher than expected in a young continent-island model. Likewise, the clustering algorithm used on BAPS 5.1 analysis grouped in the same inferred population, field populations from Scandinavia and Iceland (CNor+SENor+IceH+FIN) suggesting that those populations undergo a dynamic exchange of migrants. BAPS 5.1 migration analysis provided high Bayesian admixture estimates in both directions between Icelandic and Scandinavian populations. Supporting BAPS 5.1 results, the lowest value in the analysis of pairwise Gst, 0.063, was found between Icelandic and Scandinavian populations. Therefore, we conclude that *R. secalis* populations from Scandinavia and Iceland are following a continent-island model closer to the coalescent point than the starting point. Furthermore, Icelandic and Scandinavian

populations can be considered one inferred region, Northern Europe. In our study, Northern Europe displayed the highest diversity values. Therefore, our results supported the hypothesis in which Northern Europe represents the center of origin of *R. secalis* (Zafarano et al., 2009 and Linde et al., 2009). Because of the huge geographic barrier separating Iceland and Scandinavia, it is not expected that putative ascospores could be responsible for migration events. Therefore we conclude that the *R. secalis* migration between Iceland and Scandinavia is mediated by humans, probably, through the movement of infected seeds.

In contrast to Iceland, Switzerland is located approximately 1300km away from *R. secalis* proposed center of origin. Even if the distance between Switzerland and Scandinavia is shorter and both of them are located in the continental area, Switzerland displayed the highest pairwise Gst values. Moreover, in the Bayesian admixture analysis, Switzerland was not found as a receptor of migrants. Switzerland is connected to the migration scheme as a donor of migrants to Spain. Two different hypothesis could explain that no migrants are being introduced into Switzerland: i) a geographic isolation pattern generated by the Alps or ii) a strong purifying selection pressure imposed by the environment and/or disease management strategies as control of barley seed movement. However further research is needed to clarify these findings.

According to our migration analysis, the Spanish region was the connecting point between, Switzerland and Middle East. It is not known when *R. secalis* was first introduced into Spain. As reviewed by Moralejo et al. (1994), remains of cultivated barley have been found in the Mediterranean coast of Spain and dated to 4500 and 5590 B.C. However, Molina-Cano *et al.* (1999) hypothesized that ancient domestication of Barley could have occurred on the Iberian Peninsula and Southern France in Neolithic times, independent from the genome sources from the Fertile Crescent. Because of the strategic geographical and historical position of Spain and based on the results of our study, we hypothesize that Spanish *R. secalis* populations could play an important role in the human mediated migration processes. The Bayesian estimates of the migration between Spain and Middle East are bidirectional indicating possible movement of inoculum.

The populations of *R. secalis* sampled in Ethiopia displayed intermediate values for most of the genetic diversity indexes calculated in our study. When it comes to barley genetic

diversity, Ethiopia has been proposed as a center of origin (Vavilov, 1926) or a center of diversity (Harlan, 1971). Taking advantage from these genetic resources, worldwide barley breeding programs have been located in this country (Lakew *et al*., 1997). Several reports describe a dynamic research effort between Scandinavian countries and Ethiopia regarding barley improvement (Demissie and Bjørnstad, 1996; Bjørnstad *et al*., 2004: Grønnerød *et al*., 2002). In this frame, barley planting material has been exchanged between those countries. Further analyses of Ethiopian-Scandinavian genetic flow let us precise the field populations connecting those regions, namely CNor and ET03O. Demissie and Bjørnstad (1996) mention than in the research station of Kvithamar (sample site for CNor population) land races from Tigray (simple site for ET03O) were planted years before our sampling. In the context of dispersion means of *R. secalis*, the human mediated migration hypothesis explains long distance movement not only across the Norwegian see, but also between Africa and Europe.

It is generally assumed that the scenario of recent population expansion and the scenario of equilibrium between migration and genetic drift have contrasting effects on the pattern of spatial apportionment of genetic variation (Hamilton, 2009). In the case of species like *R secalis*, the restricted dispersal abilities limit the population expansion, therefore we could expect a correlation between the genetic distance between pairs of populations and the geographical distance that separates them, this is the so-called isolation by distance (IBD) pattern. In the study of Zaffarano *et al*. (2006) the existence of IBD patterns in *R. secalis* was investigated. Based on a global dataset and using RFLP markers, Zaffarano *et al*. (2006), found negative correlation between geographic distance and the average number of migrants per generation in *R secalis*. Differentiation between the ancestral populations and the descendant populations will decrease as the age of the descendant population increases (Slatkin 1993). Thus, it may be possible to identify a gradual range expansion by looking at the effect that the distance from the ancestral population has on genetic differentiation. We studied the IBD comparing the pairwise distance difference among population with the genetic differentiation values. We found that as the distance increases, the differentiation increases, consistent with the IBD pattern. Given that Icelandic populations did not fit with the classical island-continent model and showed high similarity with Scandinavia despite of the geographic distance among them, we wanted to know if the Icelandic populations were biasing the result of the mantel test to a landscape of no isolation by distance. When the mantel analysis was made without populations from

Iceland, the correlation was positive with a smaller p-value (0.001) indicating a better adjustment to the IBD model. Therefore, we conclude that the populations of *R. secalis* studied here have not reached the equilibrium between migration and genetic drift, indicating a recent evolutionary process. In some specific cases like Iceland, the IBD pattern has been strongly altered. We consider those cases to be associated with regions that display high interchange of infected seed material mediated by humans. Our findings are consistent with previous reports suggesting that a recent expansion process combined with infrequent long distance dispersal events have shaped the current *R. secalis* population structure (Zaffarano et al., 2009; Linde et al., 2009).

It is widely accepted that host population dynamic plays a key role in the pathogen population structure (Poulin, R., 2007). Nowadays, it is considered that barley was first domesticated in the fertile crescent in the near east and spread throughout the European continent, with movement of civilizations from the Fertile Crescent and the initiation of agricultural trade routes (Harlan, 1978). A selection process for desirable characteristics took place, resulting in the development of individual barley landraces adapted to each given area. It is likely that landraces developed in several regions were rarely, if ever, mixed until trade routes evolved from local settlements to distant sections of the world (Newman and Newman, 2008). Landraces were the source germplasm used for most of the regional breeding programs to produce the majority of barley cultivars currently used. This is also supported by recent analysis of molecular diversity and population structure of cultivated barley germplasm. Those studies advocate that barley population is highly structured by geographic region and agronomic traits (Malysheva-Otto *et al*., 2006). It was not possible for us to obtain further information regarding the genetic composition of the barley sampled for our study, however, based in the hypothethic situation explained previously, it could be considered barley cultivars from each region have high identity, and additionally, cultivars among regions could express high genetic differentiation. In this scenario, barley populations are highly structured by region, therefore a significant selection pressure over *R. secalis* populations could exist. If so, it is expected that *R. secalis* populations might also be highly structured. However, *R. secalis* populations have shown low levels of genetic differentiation among each other. It suggests that, despite of diversifying pressure ejected by the host, the suggested sexual reproduction of the pathogen and dispersion due to human mediated migration among barley fields might be disrupting the natural diversifying evolution of *R. secalis* populations.

R. secalis population structure could also be influenced by the interaction with the host resistance patterns. The most frequently studied interactions between *R. secalis* and barley are the gene-for-gene interactions between the pathogen's avirulence genes and the resistance genes of the plant. A small family of necrosis-inducing peptides (NIPs) has been identified in filtrates of *R. secalis* and in infected susceptible plants (Wevelsiep et al., 1991). The product of the gene NIP1 was also found to elicit a defense response on barley plants carrying the Rrs1 resistance gene (Rohe et al., 1995) but peptides NIP2 and NIP3 have not been found to elicit a resistance response (Hahn et al., 1993). Rohe et al. (1995) found that all avirulent isolates of *R. secalis* on Rrs1 carried the NIP1 gene, whereas the virulent isolates appeared to either lack the gene or contain an allele with a single nucleotide substitution resulting in elicitor inactivity of the gene product. At least fourteen major resistance genes to *R. secalis* have been identified in barley (Garvin et al., 1997, 2000; Jorgensen et al., 1993). None of these genes have been cloned and for most barley cultivars, it is not possible to know which resistance gene or genes they contain (Schurch et al., 2004). A study of the genetic history of *R. secalis* using the phylogenetic haplotype clustering of the avirulence gene *NIP1* and its flanking regions, showed a distribution of *R. secalis* haplotype diversity declining from north to south. This north-to-south colonization scenario reinforce the idea that *R. secalis* was originated in northern Europe. However, it is still not known, at the light of our knowledge, which has been the evolutionary pattern of the *NIPs* genes through current worldwide *R. secalis* populations, therefore we propose this as a subject of further research.

Combining our findings with previews reports, we conclude that *R. secalis* migration pattern corresponds to a mixed dispersal model. We hypothesize that ancestral *R. secalis* populations were dispersed following a stepping stone model where i) the putative ascospores linked barley field separated by only a few kilometers and/or ii) by the movement of infected seeds by Neolithic humans. The signatures of this process can be identified in the IBD pattern showed by the distribution of the diversity across the actual *R. secalis* populations. More recently, with the advent of fast and long distances transportation, the evolving process to reach a migration – drift equilibrium is being boosted. This hypothesis is supported by the finding that populations from Iceland and Scandinavia, distantly located from each other, are much more genetically connected

than Swiss and Scandinavian populations that are the geographically closer between them.

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