

Airborne Nomadic Pathogens: Does Virulence Accumulate along the Way from Paris to Beijing?

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Abstract

It is well established that race-specific resistance selects for the specifically matching genes effecting virulence in the pathogen. As well, the use of different *R*-genes in time makes virulence complexity, VC (the number of virulences per pathogen genotype), to increase. Moreover, it becomes obvious here that the wind-dispersed cereal rusts and mildews are obligate nomads and highly mobile. As a consequence of these points and as a result of modelling, VC was expected to increase in the direction of predominant winds, e.g. from west to east across Europe, and it did by approximately one or more virulences per 1000 km. Starting from the barley mildew pathogen, comprehensive evidence is also available from leaf rust on wheat covering some 5000 km from Western Europe into Siberia. The impact of our findings for population genetics across Europe and Asia is supposed to be considerable and worth further elucidating, e.g., within the 6th EU Framework Programme.

Keywords: disease resistance; population biology; host pathogen systems; wind dispersal; nomadic species; gene flow

INTRODUCTION

Cereal rusts and mildews are important wind dispersed diseases affecting crop production around the globe (OERKE *et al.* 1994; LIMPERT & BARTOŠ 1997; HAU & DE VALLAVIEILLE-POPE 1998). Among them, the barley mildew pathogen caused by *Blumeria (Erysiphe) graminis* f.sp. *hordei*, as well as the leaf rust pathogen on wheat, *Puccinia triticina*, have been extensively studied (BARTOŠ *et al.* 1996; PARK & FELSENSTEIN 1998; LIMPERT *et al.* 1999; MESTERHÁZY *et al.* 2000). It is worth re-mentioning here that they are obligate parasites on annual hosts. Being endemic over vast geographic areas covering several continents contributes to the diversity present in the populations.

The fungi have repeatedly shown to be able to adapt rapidly to major genes conferring resistance in the host (*R*-genes), by selection of the specifically matching genes effecting virulence according to the gene-for-gene concept and further forces such as gene flow and recombination (FLOR 1946; LIMPERT *et al.* 1996, 1999). As a consequence, virulence complexity, VC, i.e. the number of virulences per pathogen genotype, increased with time (MUNK *et al.* 1991) and, as another consequence, VC is worth considering in strategies to improve the use of host resistance and to reduce the selection of super-races.

Starting from points of interest on dispersal (ANDRIVON & LIMPERT 1992; HAU & DE VALLAVIEILLE-POPE 1998; AYLLOR & IRVIN 1999), it is our aim here to further focus on the understanding of population

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biology and dispersal and its possible relevance for strategies to use *R*-genes in time and space. To this aim, the need to carefully re-consider wind dispersal of spores and its effects on population genetics (LIMPERT *et al.* 1999, 2000; LIMPERT, unpublished) became increasingly clear. Further, we modelled the effects of dispersal from one region to the next and, finally, compared the outcome of modelling with comprehensive data on virulence complexity from across Europe up to Asia.

MATERIALS AND METHODS

VC was analysed for different pathogens using established procedures of pathotype (or race) analysis. Spores of the barley mildew pathogen were sampled and analysed according to LIMPERT *et al.* (1984, 1990), MÜLLER (1993) and MÜLLER *et al.* (1996). Samples were obtained from the atmosphere above Europe, and the composition of genotypes was determined for each region. Representative samples were obtained by driving across regions of interest for cereal production, with a jet spore sampler mounted on the car roof. Petri dishes with fresh leaf segments were exchanged after approximately 100 km, taking into account the use of barley varieties, and agroecological conditions in the region. Sampling started in Spain, following the season northward to keep the biological time of sampling constant. The sample size was close to 2000 isolates per year in the regions north of Geneva considered here. The virulence spectrum of individual genotypes that developed after incubation was analysed on leaf segments on agar under controlled conditions on differential sets consisting of near-isogenic lines (KØLSTER *et al.* 1986).

Analyses of the leaf rust pathogen are similar, and published results were further evaluated here. In the investigation of PARK and FELSENSTEIN (1998), the above methods for barley mildew had been further developed and adapted to the needs for leaf rust.

Whereas they used 20 near isogenic lines with different *Lr* genes (NILs) plus five differential cultivars, 15 NILs were used by MESTERHÁZY *et al.* (2000) who studied field samples.

RESULTS AND DISCUSSION

One result of importance comes from re-considering the effects of wind dispersal for life and population genetics of different species and groups of species, which becomes evident from Table 1. Whereas life is quite sedentary for the mushroom and pathogen *Armillaria*, the cereal rusts and even more so the cereal mildews (LIMPERT & BARTOŠ 1997) are obligate nomads and highly mobile.

As the air above Europe moves some 10 km/h eastward on average (LIMPERT *et al.* 2000), we modelled the effects of predominant wind and obligate mobility for population genetics and VC over three successive regions. Under the conditions mentioned in Figure 1 and with one selection cycle of the pathogen per region VC increased considerably, from 1.5 virulence genes originally to 2.25.

In addition to the model, there are a number of data sets all demonstrating consistently the increase of VC in the direction of major dispersal. Early observations of the barley mildew pathogen across Europe go back to the beginning of the 1980s (LIMPERT *et al.* 1999; LIMPERT, unpublished). During an initial phase, Western and Central Europe were thought to be one unit of population genetics (LIMPERT *et al.* 1996) but we learned that it is worth separating Great Britain from continental Europe, as both areas independently exhibit the phenomenon described (LIMPERT *et al.* 2000; unpublished).

Impressive data sets from further pathogens are available from the literature. In addition to historical data on leaf rust from Eurasia (CHESTER 1946; LIMPERT *et al.* 2000) the VC of rust isolates in Europe was most recently studied in 1998 when enough data

Table 1. Wind dispersal and population genetics: keys towards improved understanding

Pathogen	Survival strategy and wind dispersal	Conclusions for life and population genetics
1. <i>Armillaria</i> sp.	mycelium perennial – dispersal non obligate	sedentary
2. Smuts Ergot on rye	wind dispersal obligate – dispersal once per year only	obligate nomade, mobility low
3. Cereal mildews and rusts	wind dispersal obligate – dispersal several times per year	obligate nomade, mobility high

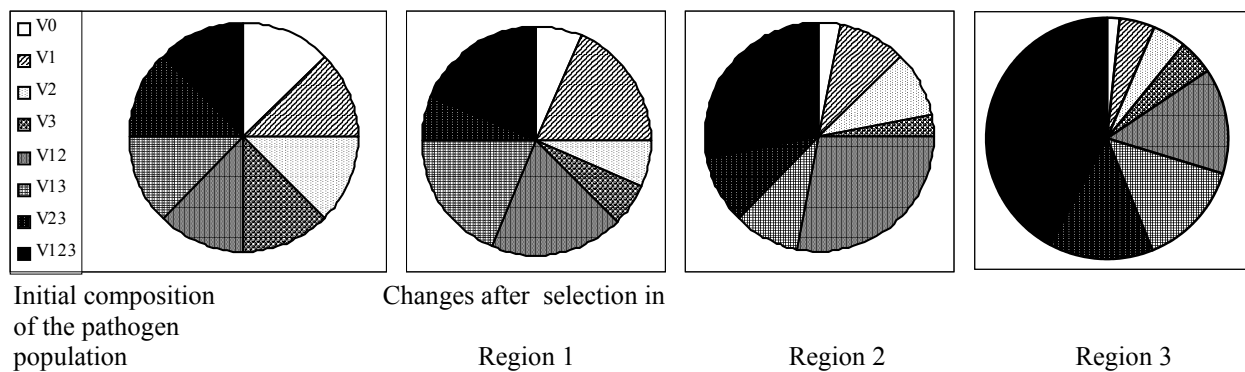


Figure 1. Modelling the effects of wind dispersal and selection on virulence complexity, VC, of a nomadic pathogen. Indicated is, from left to right, the initial composition of the pathogen population consisting of eight pathotypes with a frequency of 12.5% each, and changes after passing from one region to the next. Half of the host is sensitive, the remainder resistant, with *R*-gene 1, 2 or 3 present in region 1, 2 or 3, respectively

from 5 countries were available (MESTERHÁZY *et al.* 2000). Based on predominant pathotypes, VC was as follows. In France, 56.0% of the sample consisted out of four pathotypes with VC from 1–3, one pathotype with one gene for virulence, two with two genes and another one with three genes for virulence. In Poland, 62.3% of the samples consisted out of five pathotypes with VC ranging from 6–8, one pathotype with six virulence genes, one with seven genes and three with eight virulence genes. In Hungary, 52.5% of the samples were made from five pathotypes with VC from 4–10, one pathotype with four virulence genes, one with six genes, two with eight and one pathotype with 10 virulence genes.

In Italy, 57.4% of the sample consisted out of three pathotypes with VC from 3–6, one pathotype with

three genes, one with four genes and one with six virulence genes. Finally, in Bulgaria, 33.6% of the samples consisted of nine pathotypes with VC from 6–13, one with six virulence genes, one with seven, four with eight, two with twelve and one pathotype with thirteen virulence genes. Thus, the predominant pathotypes were rather simple in France, whereas they were increasingly complex towards the east and the south. In addition to the west-east gradient mentioned, a north south gradient of increasing VC is obvious here as well.

Most impressive are recent data from leaf rust of wheat from the area north of Geneva, where VC appears to increase quite steadily from below four in western France to above fourteen in Siberia close to Omsk (Figure 2).

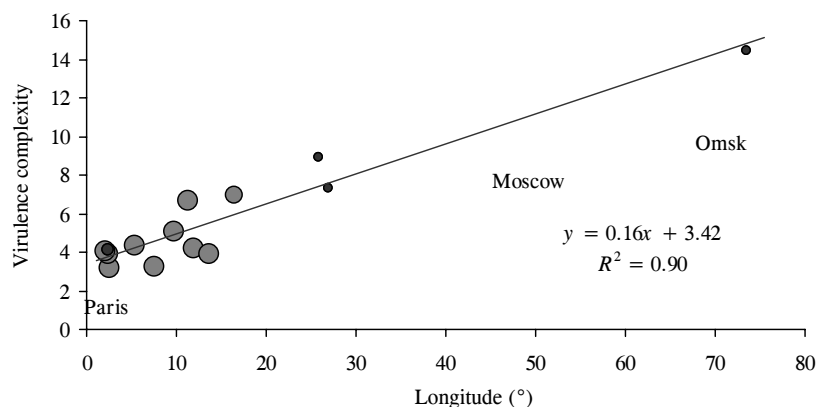


Figure 2. Leaf rust of wheat: increase of virulence complexity (VC) from west to east across Eurasia. Indicated are mean VCs of random samples from the atmosphere from mobile sampling from France to Austria (big circles) and from field sampling (small circles). Interestingly, regressions of both ways of sampling, though not indicated separately here, are very similar. Data after PARK and FELSENSTEIN (1998) re-evaluated

Although most data are from the beginning of the Eurasian wind path, the effects on population genetics and gene flow for the entire area appear to be evident and convincing. Of course, there is a considerable way left from Omsk to Beijing. However, due to major conditions remaining the same, i.e. predominating winds from the west and presence of the host, the conclusion of virulence genes accumulating along the way from Paris to Beijing appears to be justified. Further data of interest for the concept come from crown rust of oats caused by *Puccinia coronata* (ŠEBESTA *et al.* 1999) and are currently re-evaluated.

CONCLUSIONS AND OUTLOOK

The hypothesis becoming obvious from the title is well supported by modelling, comprehensive data and logic. Our answer to the title question is positive, thus giving birth, amongst others, for a *Eurasian path of population genetics* along which genes and genotypes move. There are important conclusions for disease resistance and, for instance, the question on costs of unnecessary virulence that are described elsewhere (LIMPERT & BARTOŠ 2003).

Obviously, in addition to wind further factors such as virulence combinations, climate and temperature or alternate hosts can also affect VC in various ways and would benefit, thus, from further analyses. Warmer climate for instance is more favourable for wheat leaf rust in eastern Europe, whereas the opposite is true for cereal mildews. Moreover, the co-evolutionary processes with epidemic dynamics described in natural populations (BURDON & THRALL 2002) would also be worth studying for the effect on VC, both from the regional to the pandemic scale.

Let us finish with a most general conclusion. Presumably, there is no other biomass production around the globe as big and genetically uniform as that of cereals. Consequently, this should be true also for the biomass of their pathogens. The cereal pathogens are thus supposed to have a pilot function for the recognition of host-pathogen interactions in general, and even beyond that. Indeed, the rules of population genetics described here should be applicable for a wide scope of species including insects, spiders and even some crustaceae.

Fascinating challenges should thus be in front of us, not only leading to new views about disease resistance in plant pathology, but also to novel insight into geo-phytopathology, geo-biomedicine including veterinary and human medicine and geo-biology, and

could thus give rise to further knowledge on important aspects of, e.g., foot and mouth disease, influenza and Malaria (SHORTTRIDGE & STUART-HARRIS 1982; DONALDSEN *et al.* 2001). To this aim, we recently submitted an Expression of Interest to the European Union entitled *Effects of Wind and Bio-Aerosols on Health and Growth across Europe*, to which further thoughts are always welcome.

References

- ANDRIVON D., LIMPERT E. (1992): Origin and proportions of components of populations of *Erysiphe graminis* f.sp. *hordei*. J. Phytopathol., **135**: 6–19.
- AYLOR D. E. IRVIN M.E. (eds) (1999): Aerial dispersal of pests and pathogens. Special Issue, Agric. For. Meteorol., **97**: 233–252.
- BARTOŠ P., STUHLÍKOVÁ E., HANUŠOVÁ R. (1996): Adaptation of wheat rusts to the wheat cultivars in former Czechoslovakia. Euphytica, **92**: 95–103.
- BURDON J.J., THRALL P.H. (2002): Resistance variation in natural plant populations. Proc. 6th Conf. EFPP 2002, Prague, Plant Protect. Sci., **38** (Special Issue 1): 139–144.
- CHESTER K.S. (1946): The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat. Chronica Botanica, Waltham, Massachusetts.
- DONALDSEN A.I., ALEXANDERSEN S., SØRENSEN J.H., MIKKELSEN T. (2001): Relative risks of the uncontrollable (airborne) spread of FMD by different species. Vet. Record, **148**: 602–604.
- FLOR H.H. (1946): Genetics of pathogenicity in *Melampsora lini*. J. Agric. Sci., **73**: 335–337.
- HAU B., DE VALLAVIEILLE-POPE C. (1998) Wind-dispersed diseases. In: JONES D.G. (ed.): The Epidemiology of Plant Diseases. Kluwer Publishers, Dordrecht: 323–347.
- KØLSTER P., MUNK L., STØLEN O., LØHDE J. (1986): Near-isogenic barley lines with genes for resistance to powdery mildew. Crop Sci., **26**: 903–907.
- LIMPERT E., BARTOŠ P. (1997): Analysis of pathogen virulence as decision support for breeding and cultivar choice. In: HARTLEB H., HEITEFUSS H., HOPPE H.-H. (eds): Resistance of Crop Plants against Fungi. Fischer, Jena: 401–424.
- LIMPERT E., BARTOŠ P. (2003): Wind-dispersed nomadic pathogens – conclusions for disease resistance. Czech J. Genet. Plant Breed., **38** (in press).
- LIMPERT E., SCHWARZBACH E., FISCHBECK G. (1984): Influence of weather and climate on epidemics of barley mildew, *Erysiphe graminis* f.sp. *hordei*. In: LIETH H., FANTECHI R., SCHNITZLER H. (eds): Interaction between

- Climate and Biosphere. Progress in Biometeorology, 3, Swets & Zeitlinger B.V. Lisse: 146–157.
- LIMPERT E., ANDRIVON D., FISCHBECK G. (1990): Virulence patterns in populations of *Erysiphe graminis* f.sp. *hordei* in Europe in 1986. Plant Pathol., **39**: 402–415.
- LIMPERT E., FINCKH M.R., WOLFE M.S. (eds) (1996): Integrated control of cereal mildews and rusts: towards co-ordination of research across Europe. In: Proc. Workshop COST 817, European Commission, Luxembourg.
- LIMPERT E., GODET F., MÜLLER, K. (1999): Dispersal of cereal mildews across Europe. Agric. Forest Met., **97**: 293–308,
- LIMPERT E., BARTOŠ P., GRABER W.K., MÜLLER K., FUCHS J.G. (2000): Increase of virulence complexity of nomadic airborne pathogens from west to east across Europe. Acta Phytopath. Entomol. Hung., **35**: 261–272.
- MESTERHÁZY Á., ANDERSEN O., BARTOS P., CASULLI F., CSÖSZ M., GOYEAU H., ITTU M., JONES E., MANISTERSKI J., MANNINGER K., PASQUINI M., RUBIALES D., SCHACHERMAYER G., STRZEMBICKA A., SZUNICS L., TODOROVA M., UNGER O., VANCO B., VIDA G., WALTHER U. (2000): European virulence survey for leaf rust in wheat. Agronomie, **20**: 793–804.
- MÜLLER K. (1993): Virulenzstruktur und -Dynamik grossräumiger Populationen des Gerstenmehltaus (*Erysiphe graminis* f.sp. *hordei* Marchal) in Europa. [PhD. Thesis ETHZ 10008].
- MÜLLER K., MCDERMOTT J.M., WOLFE M.S., LIMPERT E. (1996): Analysis of diversity in populations of plant pathogens: the barley powdery mildew pathogen across Europe. Eur. J. Plant Pathol., **102**: 385–395.
- MUNK L., JENSEN H.P., JØRGENSEN J.H. (1991): Virulence and disease severity of barley powdery mildew in Denmark. In: JØRGENSEN J.H. (ed.): Integrated Control of Cereal Mildews: Virulence Patterns and their Change. Proc. 2nd Europ. Workshop on Integrated Control of Cereal Mildews, Risø National Laboratory: 55–65.
- OERKE E.C., DEHNE H.W., SCHÖNBECK F., WEBER A. (1994): Estimated Losses in Major Food and Cash Crops. Elsevier, Amsterdam.
- PARK R.F., FELSENSTEIN F.G. (1998): Physiological specialization and pathotype distribution of *Puccinia recondita* in western Europe, 1995. Plant Pathol., **47**: 157–164.
- SHORTRIDGE K.F., STUART-HARRIS C.H. (1982): An influenza epicentre? Lancet, **2**: 812–813.
- ŠEBESTA J., ZWATZ B., RODERICK H.W., HARDER D.E., STOJANOVIĆ S., CORAZZA L. (1999). Biological (Genetic) control of fungal diseases of oat in Europe. Pflanzenschutz Ber., **58**/Heft 2: 152.