

# Immunogenetics and hantaviruses

From genes to the assesment of emergence risk

N Charbonnel,

M Galan, J Bryja, PJ Malé, E Guivier, Y Chaval, J Deter, S Morand, JF Cosson



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What explains the intra- and inter-specific variability in susceptibility to zoonotic agents ?



**Genetic attributes of the host are important**, especially immune genes :

Immune genes are known to influence the outcomes of Host-Pathogen interactions

e.g. intraspecific for humans: malaria, HIV, hepatitis, bilharziosis, ...

**Major applications =** {  
• Immunotherapeutics and design of vaccines  
• Detection of resistance in livestock

But, ...

## Background : 1-Immunogenetics in Eden

Another less developed **application of immunogenetics**

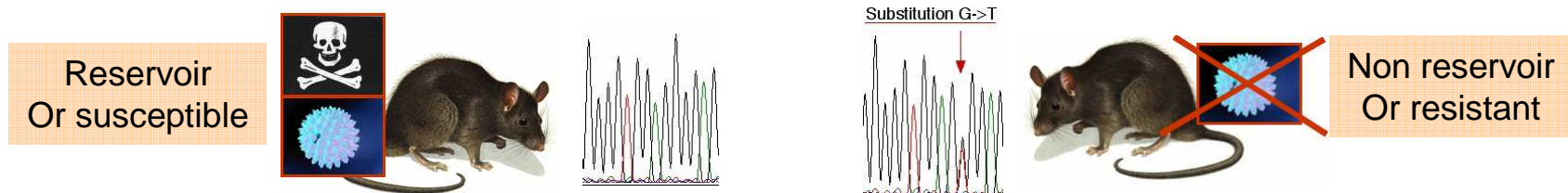
= the assessment of emergent or re-emergent **disease risks** in natural populations

↪ A reason why hosts differ in their susceptibility/resistance to pathogens could rely on the degree of matching between immune genes and pathogen antigens

- Between species : Which species can be a reservoir and which one can not ?

- Within species : Are there resistant / susceptible individuals for a given pathogen?

⇒ Better assesment of epidemiology, important for modelling



Within species, the **distribution** of mutations at these target immune genes informs

At local scale: can zoonotic agents spread, persist locally ?

Immunogenetics could be included in **epidemiological risk modelling**

Across large geographical areas: where can emergence occur ?

Immunogenetics could be included in **risk mapping**

## Background : 2- Immunogenetics and hantaviruses

### In humans : Several immune genetic factors affect the severity of the infection with *Puumala virus*

VIRAL IMMUNOLOGY  
Volume 19, Number 3, 2006  
© Mary Ann Liebert, Inc.  
Pp. 558-564

Brief Report

Tumor Necrosis Factor- $\alpha$  Genetic Predisposing Factors Can  
Influence Clinical Severity in Nephropathia Epidemica

PIET MAES,<sup>1</sup> JAN CLEMENT,<sup>1</sup> PAUL H.P. GROENEVELD,<sup>2</sup> PAUL COLSON,<sup>3</sup>  
TOM W.J. HUIZINGA,<sup>4</sup> and MARC VAN RANST<sup>1</sup>

Scandinavian Journal of Immunology

Volume 47 Issue 3 Page 277-279, March 1998

Association of HLA B27 with Benign Clinical Course of  
Nephropathia Epidemica Caused by Puumala Hantavirus

- Mustonen, Partanen, Kanerva, Pietilä, Vapalahti, Pasternack & Vaheri
- <sup>1</sup>Medical School, University of Tampere and Tampere University Hospital, Tampere,  
<sup>2</sup>Tissue Typing Laboratory, Finnish Red Cross Blood Transfusion Service, Helsinki,  
<sup>3</sup>Department of Virology, Haartman Institute, University of Helsinki, Helsinki,  
Finland

843

CONCISE COMMUNICATION

Human Leukocyte Antigen-B8-DR3 Is a More Important Risk Factor  
for Severe Puumala Hantavirus Infection than the Tumor Necrosis  
Factor- $\alpha$ (-308) G/A Polymorphism

Satu Mäkelä,<sup>1,2</sup> Jukka Mustonen,<sup>1,2</sup> Ilpo Ala-Houhala,<sup>1,2</sup>  
Mikko Hurme,<sup>1,2</sup> Jukka Partanen,<sup>3</sup> Olli Vapalahti,<sup>4,5</sup>  
Antti Vaheri,<sup>4,5</sup> and Amos Pasternack<sup>1,2</sup>

<sup>1</sup>Medical School, University of Tampere and <sup>2</sup>Tampere University  
Hospital, Tampere, <sup>3</sup>Department of Tissue Typing, Finnish Red Cross  
Blood Transfusion Service, <sup>4</sup>Department of Virology, Haartman  
Institute, University of Helsinki and <sup>5</sup>HUCH Laboratory Diagnostics,  
Helsinki, Finland

JOURNAL OF CLINICAL MICROBIOLOGY, May 1997, p. 1090-1096  
0095-1137/97/\$04.00+0  
Copyright © 1997, American Society for Microbiology

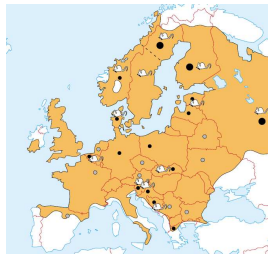
Vol. 35, No. 5

Puumala Hantavirus Genome in Patients with Nephropathia  
Epidemica: Correlation of PCR Positivity with HLA Haplotype  
and Link to Viral Sequences in Local Rodents

ALEXANDER PLYUSNIN,<sup>1\*</sup> JAN HÖRLING,<sup>2</sup> MARI KANERVA,<sup>1</sup> JUKKA MUSTONEN,<sup>3</sup> YING CHENG,<sup>1</sup>  
JUKKA PARTANEN,<sup>4</sup> OLLI VAPALAHTI,<sup>1</sup> SAMI K. J. KUKKONEN,<sup>1</sup> JUKKA NIEMIMAA,<sup>5</sup>  
HEIKKI HENTTONEN,<sup>5</sup> BO NIKLASSON,<sup>2</sup> ÅKE LUNDKVIST,<sup>2</sup> AND ANTTI VAHERI<sup>1</sup>

Immune genes, rodents and hantaviruses

A single study :



I will focus on *Puumala virus* / *My. glareolus*



Journal of Medical Virology

Volume 74, Issue 1 , Pages 180 - 190

**DIFFERENTIAL EXPRESSION OF IMMUNOREGULATORY GENES IN MALE AND FEMALE NORWAY RATS FOLLOWING INFECTION WITH SEOUL VIRUS**

Sabra L. Klein<sup>1\*</sup>, Amy Cemetich<sup>1</sup>, Sara Hilmer<sup>2</sup>, Eric P. Hoffman<sup>2</sup>, Alan L. Scott<sup>1</sup>, Gregory E. Glass<sup>1</sup>

<sup>1</sup>W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

<sup>2</sup>Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC

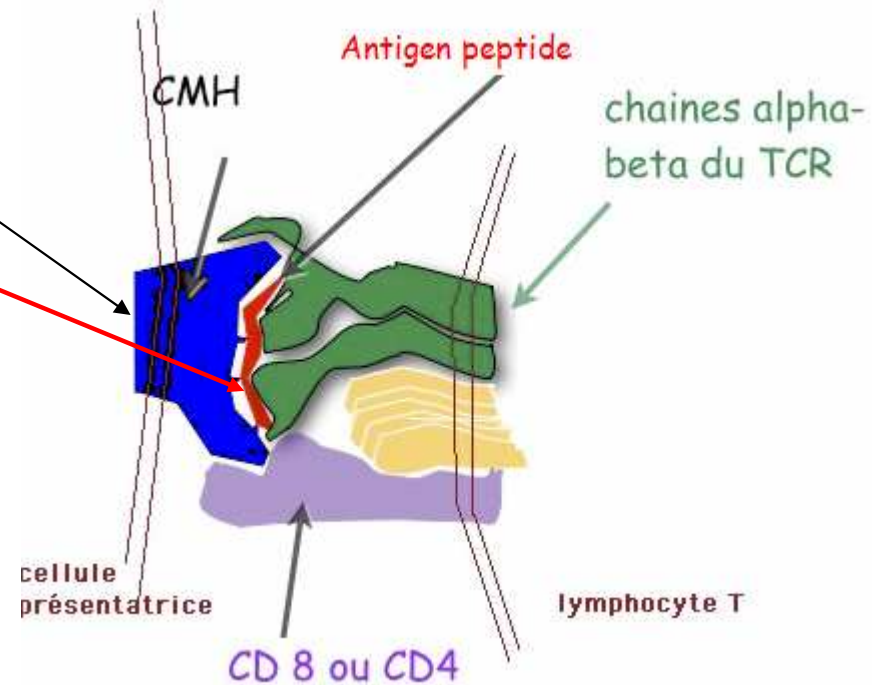
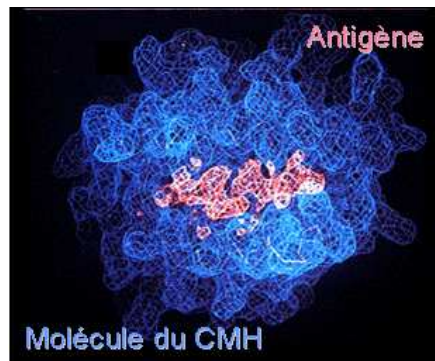
I- **Pre-requisite** : is there any evidence of selection mediated by Puuv on bank voles ? Can we identify resistant rodents from experimental infestations ?

Ila- Genetic characteristics of these 'resistant' rodents at immune genes ?

Ilb- Genetic characteristics of hantavirus seropositive vs seronegative rodents in the field at these immune genes ? Spatial variations at a local scale ?

III- At a large geographical scale, can we partly explain the distribution of *Puumala virus* from the distribution of the variability (susceptible and resistant alleles) of immune genes ?

MHC genes encode cell surface glycoproteins that present peptides of foreign proteins to T cells



### MHC gene characteristics

- Most **variable** genes known for vertebrates
- **Selection** maintains MHC gene variability
- **Parasite** mediated selection

A host with a higher number of MHC proteins may enable the recognition of a larger spectrum of parasites

Mutant pathogens can escape from host MHC molecules

## I- Pre-requisite : Selection mediated by *Puumala virus* on bank voles

**Traditionally, hantavirus** infections have been thought to be **harmless** to their **rodent hosts**.

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

169

Eva R. Kallio

Experimental Ecology on the  
Interaction between the  
Puumala Hantavirus and its Host,  
the Bank Vole

Ecology. 2007 Aug;88(8):1911-6

**Endemic hantavirus infection impairs the winter survival of its rodent host.**

**Kallio ER, Voutilainen L, Vapalahti O, Vaheri A, Henttonen H, Koskela E, Mappes T.**

Department of Biological and Environmental Science, P.O. Box 35, FIN-40014, University of Jyväskylä, Finland. [eva.kallio@evira.fi](mailto:eva.kallio@evira.fi)

The **over-winter survival** of bank voles was influenced by PUUV infection. The Puuv infected individuals had significantly **lower** probability to survive from October to May than the non-infected individuals.



# I- Pre-requisite : Resistant bank voles identified from experimental infestations

**Table 1.** Bank voles used in the *in vivo* experiments

Infected individuals (detected by RT-PCR) are in bold. RGr's are shown with the number of days since removal of the donors in parentheses. During these periods the RGr individuals were exposed to the donors' beddings. F, female; M, male. The numbers after F or M represent the age of the animals (in weeks), at which they were exposed to the donors' beddings.

Isolator	Donor individual	RGr1 (0-3)	RGr2 (3-6)	RGr3 (6-9)	RGr4 (9-12)	RGr5 (12-15)	RGr6 (15-18)	RGr7 (18-21)	RGr8 (21-24)	RGr9 (24-27)	RGr10 (27-30)	No. infected/total
1	1: F 18	F 21	F 12	F 21	F 22	F 22	-	-	-	-	-	3/5
	2: F 18	F 21	F 18	M 18	M 21	F 21	-	-	-	-	-	5/5
	3: F 15	F 18	F 21	F 21	M 19	F 19	-	-	-	-	-	3/5
	4: F 12	F 15	M 23	F 13	M 19	F 19	-	-	-	-	-	3/5
	5: F 11	F 12	F 21	F 12	M 15	F 15	-	-	-	-	-	3/5
2	6: F 11	F 12	M 12	F 18	F 21	M 21	-	-	-	-	-	2/5
	7: M 21	M 24	F 20	M 22	F 22	F 22	-	-	-	-	-	0/5
	8: M 17	M 20	F 12	M 20	M 15	F 15	-	-	-	-	-	0/5
	9: M 15	M 18	M 18	M 12	F 15	F 15	-	-	-	-	-	1/5
	10: M 15	M 12	M 20	F 12	F 16	F 16	-	-	-	-	-	0/5
3	11: F 12	F 10	F 10	F 11	F 10	F 7	-	-	-	-	-	2/5
	12: F 11	M 7	F 7	M 10	F 7	F 11	-	-	-	-	-	1/5
	13: F 11	M 9	F 11	M 8	F 11	M 10	-	-	-	-	-	3/5
	14: F 9	F 10	M 8	F 7	M 8	M 8	-	-	-	-	-	0/5
	15: F 8	F 8	M 9	F 9	M 9	M 9	-	-	-	-	-	1/5
4	16: F 17	F 21	F 19	F 14	M 20	F 15	F 13	M 18	M 13	M 14	F 10	1/10
	17: F 17	M 11	M 14	F 21	F 22	F 20	M 12	M 9	F 11	M 12	M 13	1/10
5	18: M 17	F 16	M 19	F 19	F 11	M 12	F 10	M 10	M 12	F 18	M 14	2/10
	19: M 17	M 19	*	M 11	M 11	F 11	F *	M 12	F 14	M 13	F 17	0/10
6	20: F 17	F 11	M 18	M 18	F 20	M 11	F 18	F 13	F 12	F 11	F 10	1/10
	21: M 17	M 16	F 21	M 19	M 19	M 19	M 16	F 19	M 20	F 11	M 11	0/10
Total	21/21	5/21	6/21	7/21	7/21	7/21	0/6	0/6	0/6	0/6	0/6	32/135

Infected voles

*Journal of General Virology* (2006), 87, 2127-2134

DOI 10.1099/vir.0.81643-0

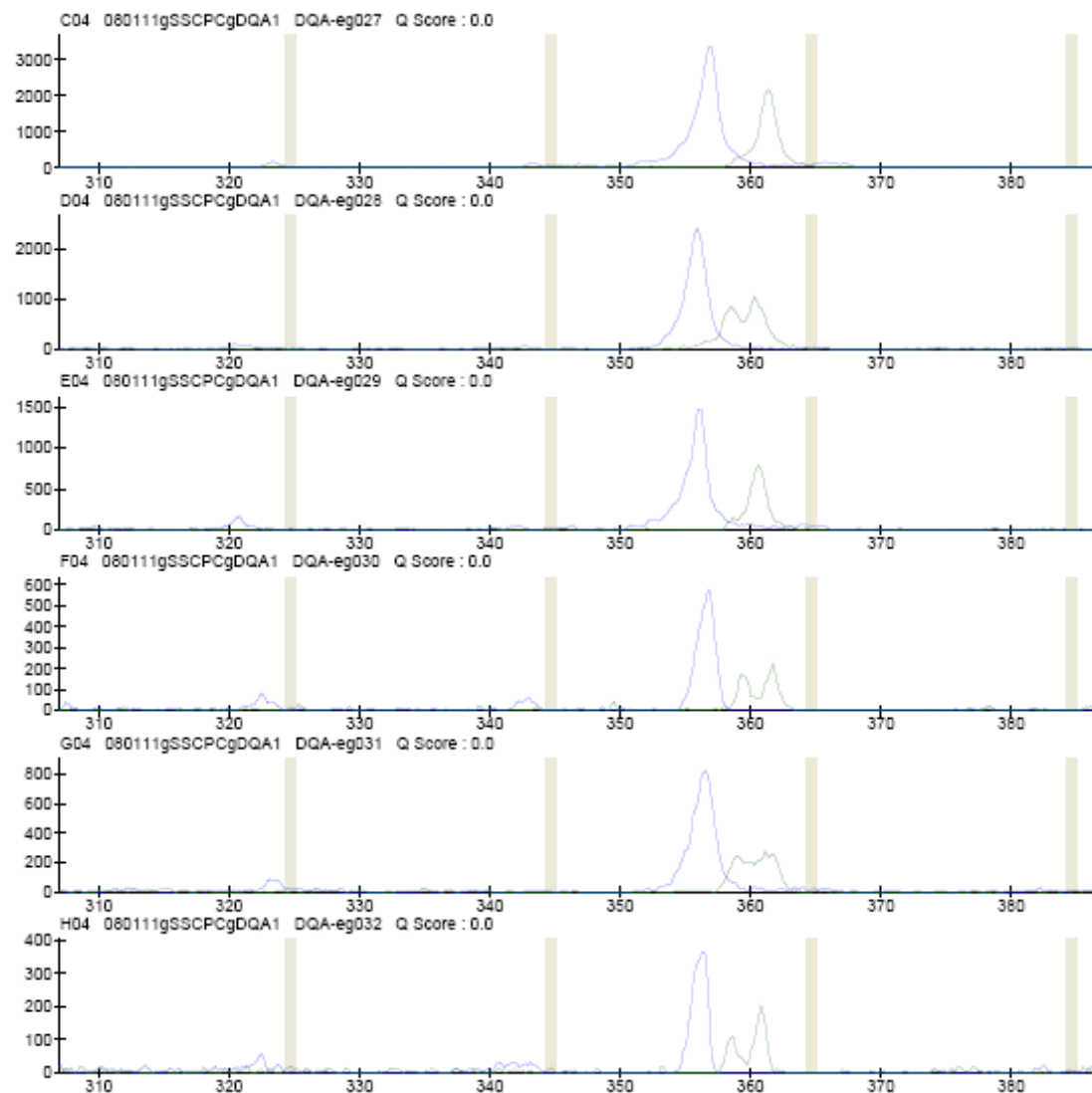
Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment

Eva R. Kallio,<sup>1,2,5</sup> Jonas Klingström,<sup>3,4</sup> Elisabeth Gustafsson,<sup>4</sup> Tytti Manni,<sup>6</sup> Antti Vaheri,<sup>6</sup> Heikki Henttonen,<sup>1</sup> Olli Vapalahti<sup>5,6,7</sup> and Åke Lundkvist<sup>3,4</sup>



## Ila- Genetic characteristics of these 'resistant' bank voles at MHC genes ?

### SSCP results on DQA exon 2 gene

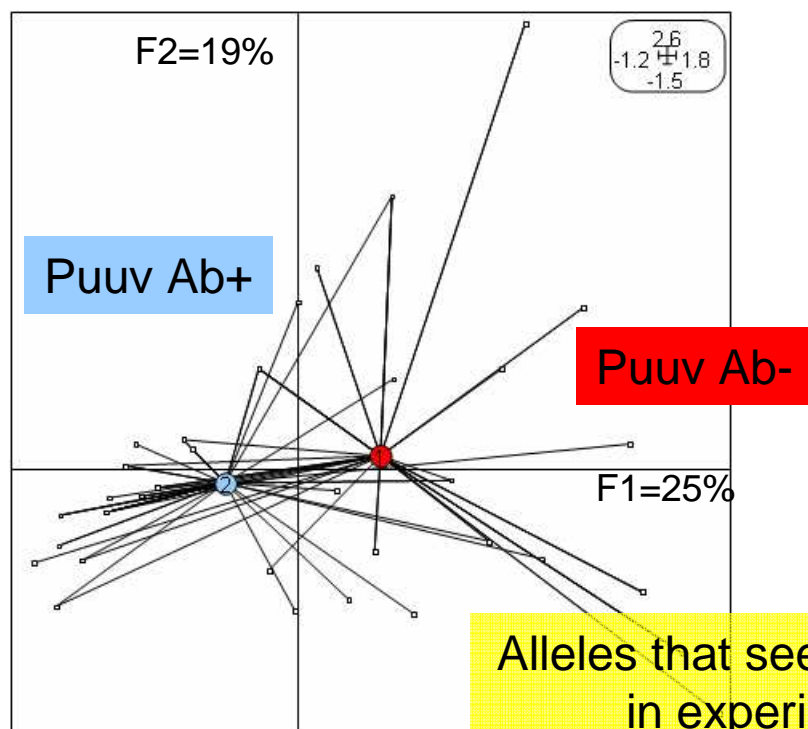


Few alleles detected

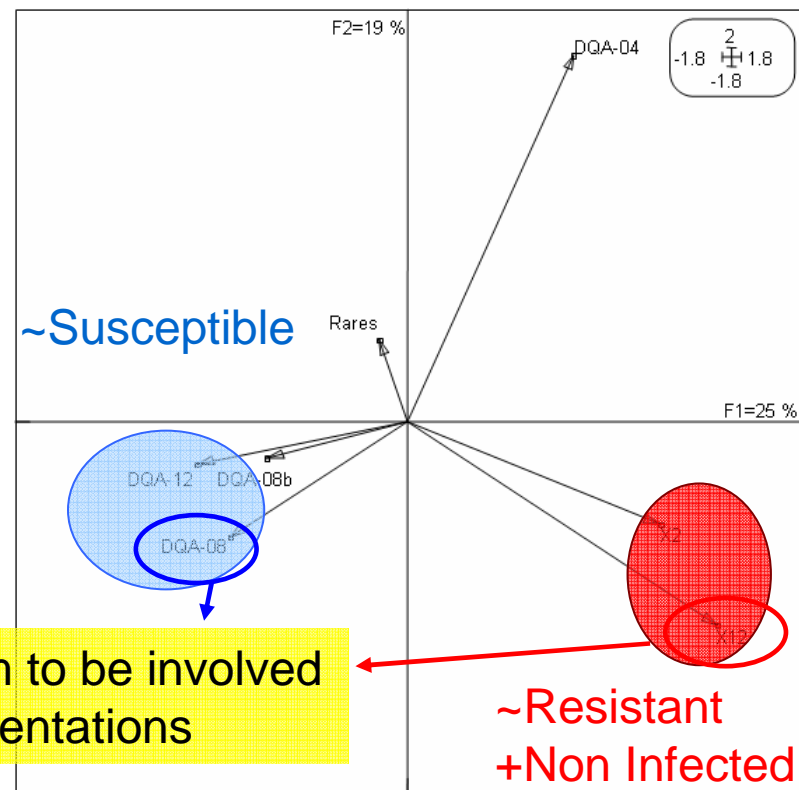
No statistical differences between infected and non-infected individuals at DQA and DRB genes  
(Guivier et al. in prep)

## IIb- Associations between Puumala-seropositivity and MHC

① Multivariate analysis of genetic dataset from two sites in finland



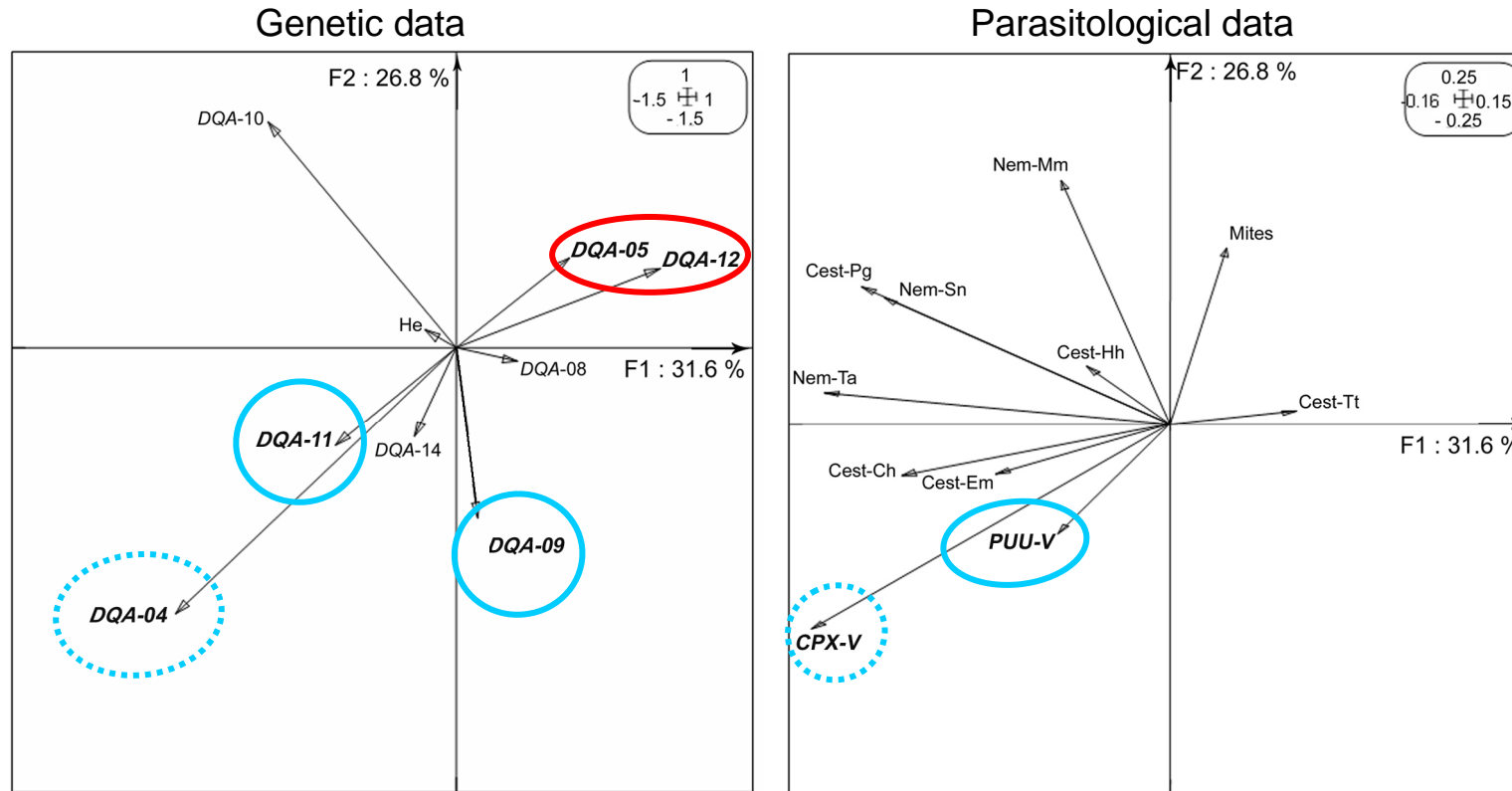
Strong differentiation between seropositive and seronegative individuals



Identification of alleles positively and negatively associated with seropositivity

## IIb- Associations between Puumala-seropositivity and MHC

② We have found associations between alleles and Puuv-Ab in France  
*(Deter et al., IGE, 2008)*



Coinertia  
 Associations  
 between MHC  
 and roboviruses

⊕ « **Susceptibility** »

Puumala / DQA09

Relative risk = 2.83

Puumla / DQA11

Relative risk = 2.07

⊖ « **Resistance** »

Puumala / DQA12

Relative risk = 0.57

Puumala / DQA05

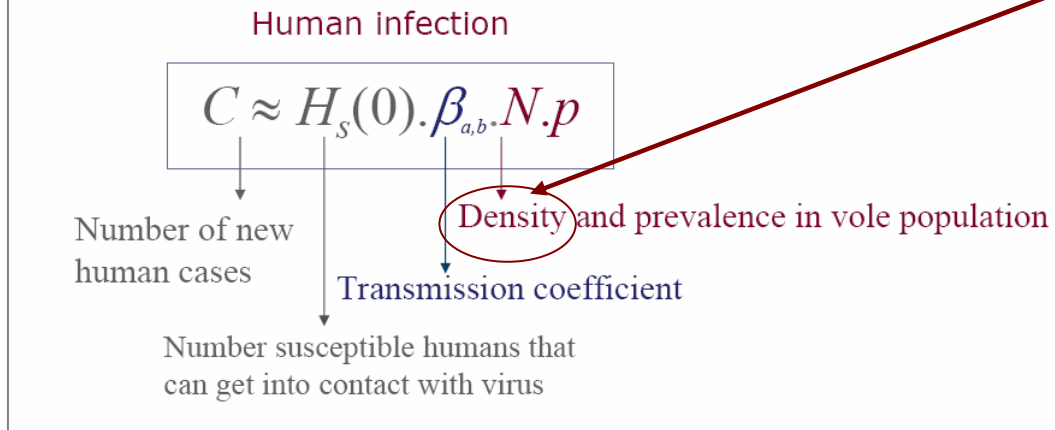
Relative risk = 0.81



## IIb- Associations between Puumala-seropositivity and MHC

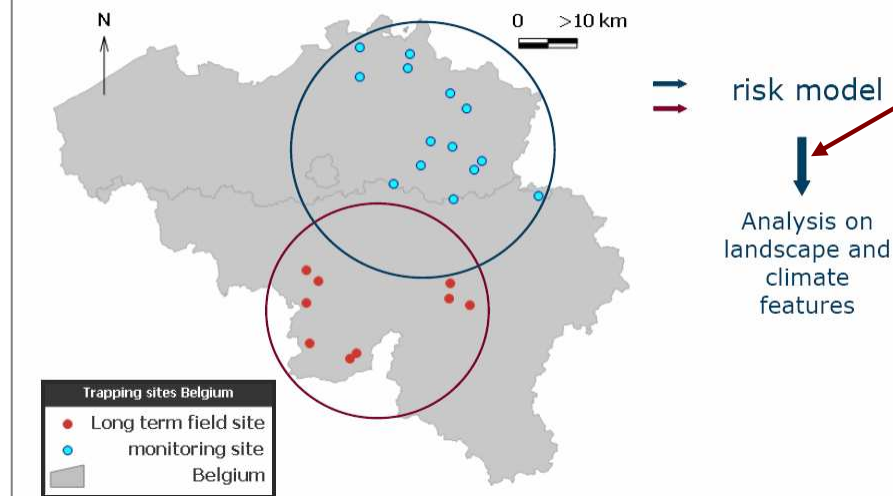
### Potential applications to modelling

#### 2. Risk factors related to Puumala infection in Belgium



Include immunogenetics :  
Resistant vs sensible voles

#### Environmental parameters related to PUUV infection in voles



Include  
Gene flow between sites  
and immunogenetics

Genotyping is done  
Analyses in progress



From K. Tersago, Eden 2007

## Genetic structure of bank vole and Puumala virus in Fennoscandia: Results

### Bank vole phylogeography

● Western lineage

● Eastern lineage

● Ural lineage

🟦 Lakes

### Puumala phylogeography

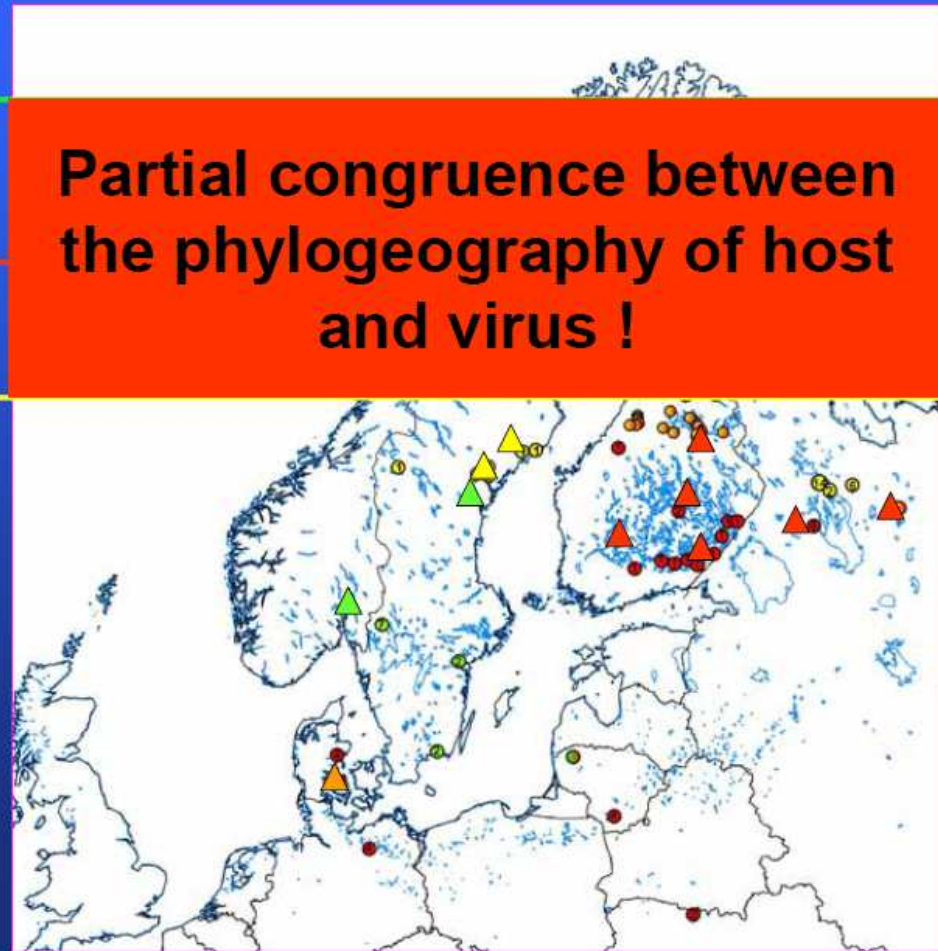
▲ S-SCA strain

▲ FIN strain

▲ N-SCA strain

▲ DAN strain

**Partial congruence between the phylogeography of host and virus !**



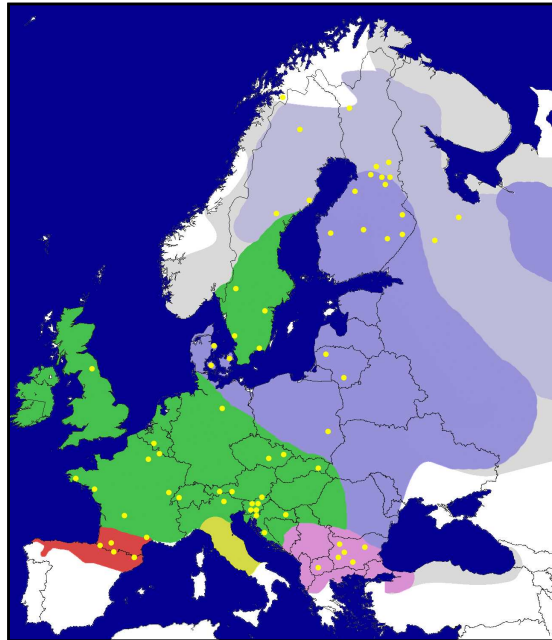
Asikainen *et al.* 2000  
Deffontaine *et al.* In preparation

Influence of the coevolution between *My. glareolus* and *Puumala virus* on immune genes ?

### III- MHC genes at a large geographical scale

3- At a large geographical scale, can we explain the distribution of *Puumala virus* from the distribution of the variability (susceptible and resistant alleles) of immune genes ?

70 localities – 382 individuals



➔ 6 Mitochondrial lineages

Analysis of molecular variance  
(AMOVA)

#### Cytochrome B (V. Deffontaine)

Differentiation within group ( $\Phi_{sc}$ ) 0.26

Differentiation between groups  
( $\Phi_{CT}$ ) 0.73

#### MHC gene

Differentiation within group ( $\Phi_{sc}$ ) 0.38

Differentiation between groups  
( $\Phi_{CT}$ ) 0.01

Phylogeographic history does not explain the distribution of MHC polymorphism



### III- MHC genes at a large geographical scale

Comparing *Puumala virus* distribution and the geographic variability of immune genes

Spatial analysis of molecular variance

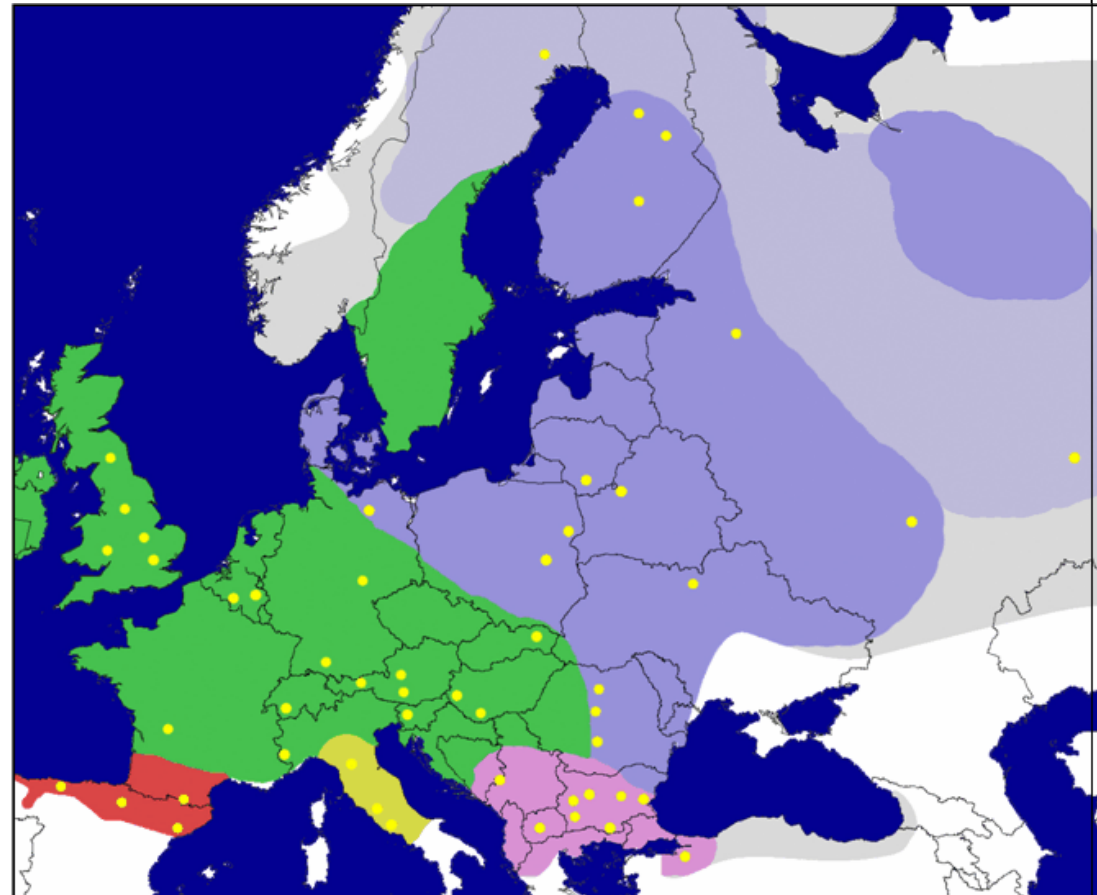
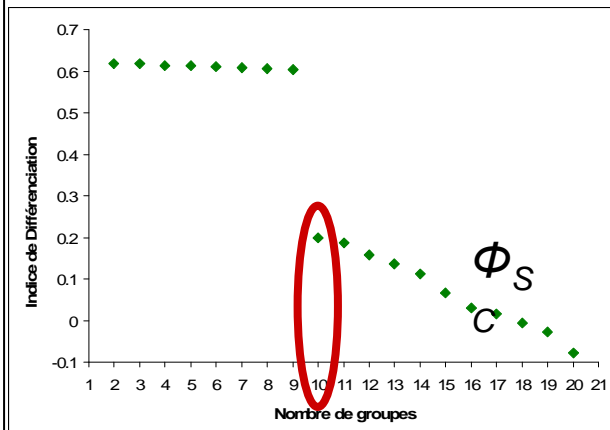
(SAMOVA)

Finds the best geographical structure



Differentiation within group ( $\Phi_{SC}$ )

**Cytochrome B**



### III- MHC genes at a large geographical scale

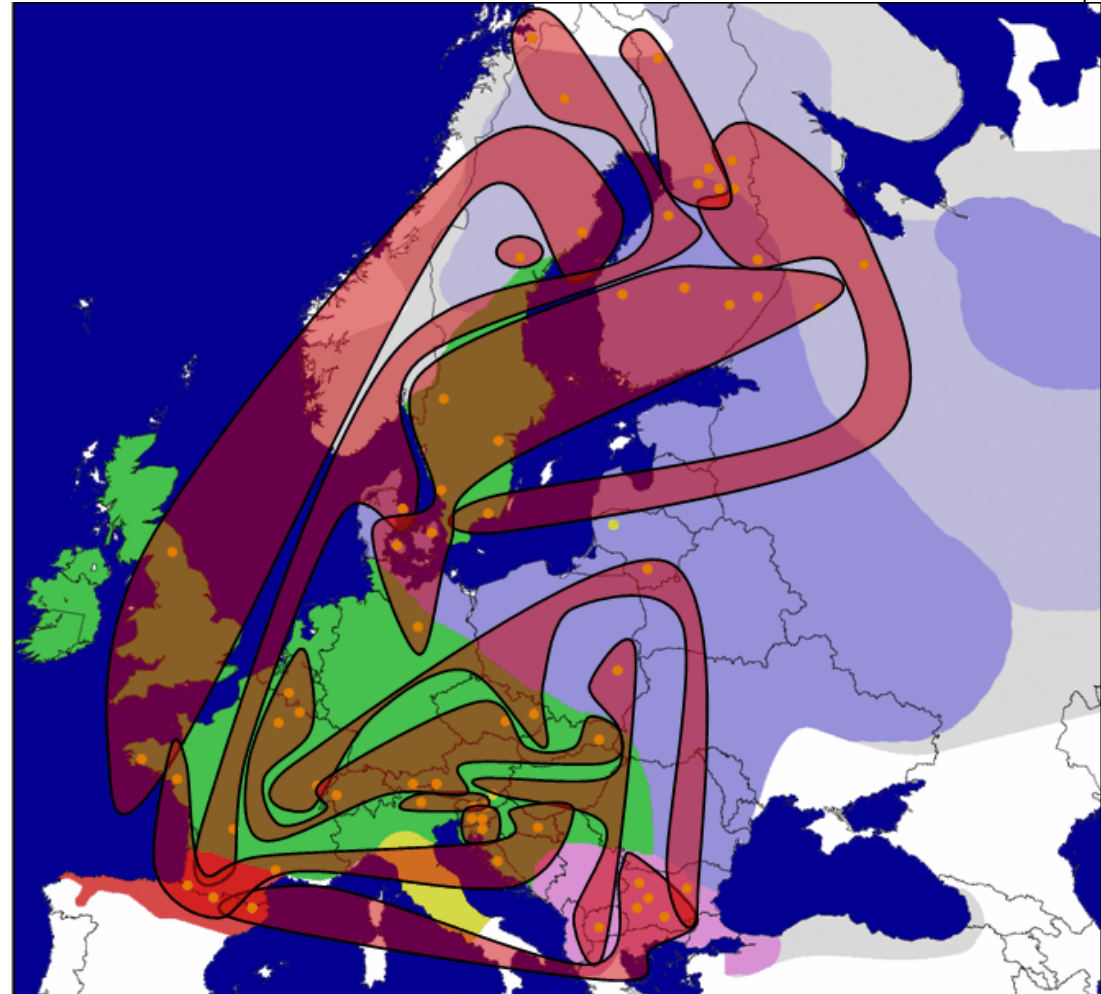
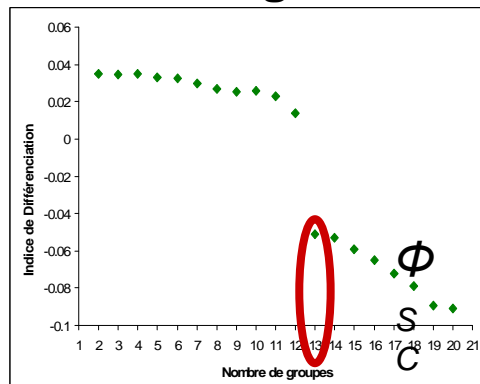
Spatial analysis of molecular variance  
(SAMOVA)

$k = 13$



No genetic structure associated with geography for the MHC gene.

**MHC gene**



### III- MHC genes at a large geographical scale

The presence of alleles does not depend on geography

MHC allele distribution does not explain  
endemic vs non endemic areas

But the frequency of alleles is not included in these analyses

could it be an important factor ?

Pbl : impossible at the moment to estimate frequencies ...

But, in the future :

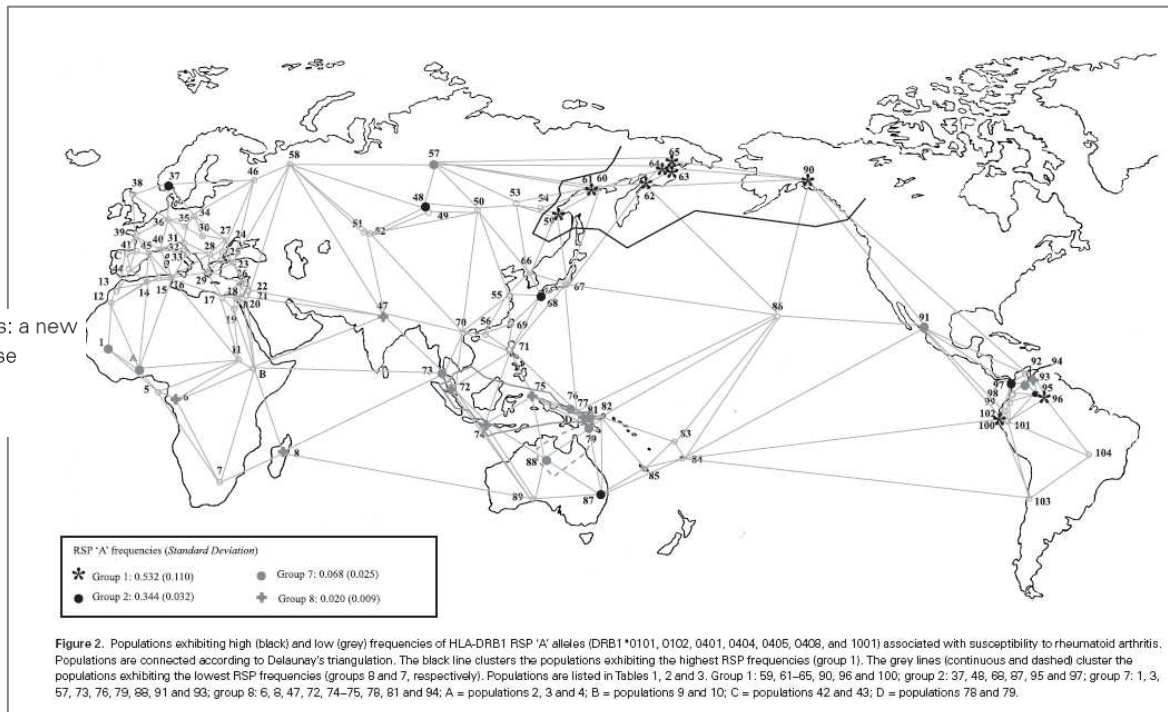
Application to risk mapping

Eg:

Geographic patterns of functional categories of HLA-DRB1 alleles: a new approach to analyse associations between HLA-DRB1 and disease

M. Gibert\*† & A. Sanchez-Mazas‡

© 2003 Blackwell Publishing Ltd, *European Journal of Immunogenetics* 30, 361–374





## Bilan

We have found associations between MHC class II gene and Puumala-Ab  
MHC class II gene could be involved (directly or not) in *M. glareolus*  
susceptibility to *Puumala virus*

At the moment, no geographic signal explaining *Puumala virus* endemicity

↪ Coevolution could lead to spatial fluctuations of selection



## Prospects

- ① Compare associations found using  
Experiments / Field
- ② Analyse spatio-temporal surveys of  
MHC polymorphism in endemic areas
  - Fluctuations ?
  - Relation with *Puumala* infections ?

Improve small scale **risk modelling**

- ③ Study other genetic factors involved  
TNF- $\alpha$ , INF, Intgr ...

Improve **large scale risk mapping**

Emmanuel Guivier PhD  
2007-2010