Emergence of `birch-leafroll disease´ in Fennoscandia correlated with significant changes in *Cherry leaf roll virus* population

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Introduction A viral epidemic associated with the *Cherry leaf roll virus* has emerged in *Betula* species in Fennoscandia exhibiting quick and effective dispersal (Jalkanen et al., 2007). We studied one natural CLRV population from Rovaniemi and one population that occurred after grafting young *Betula* seedlings with scions from the original trees to determine the virus´s diversity and population structure. The further goal of our study is to determine the disease´s causal agents and restrict its dispersal. 14 samples from naturally-infected birches (Fig. 1) and 19 samples from grafted birch seedlings with twigs originating from five CLRV-infected *B. pubescens* donor trees from Rovaniemi (Bpub3, Bpub4, Bpub7, Bpub20 and Bpub320) were used for the analysis.

Fig. 2 CLRV-specific symptoms in *Betula pubescens* seedlings grafted with scions from naturally-infected birches

Fig. 1 CLRV-specific symptoms (Büttner et al., 2013) in *Betula pubescens* leaves from naturally-infected trees in Rovaniemi (vein banding, leaf roll and deformation, chlorotic ringspots, necrosis, reduced leaf size, dwarfing).

M &M Total RNA was extracted and nested RT-PCR was performed in three genomic regions from both RNAs; the coat protein (CP), the RNA-dependent-RNA-polymerase (RdRp) and the 3´- untranslated (3´-UTR) regions. PCR products were cloned and five independent colonies from each ligation event were sequenced. Bootstrapped Maximum Likelihood (ML) phylogenetic trees were constructed with MEGA6.

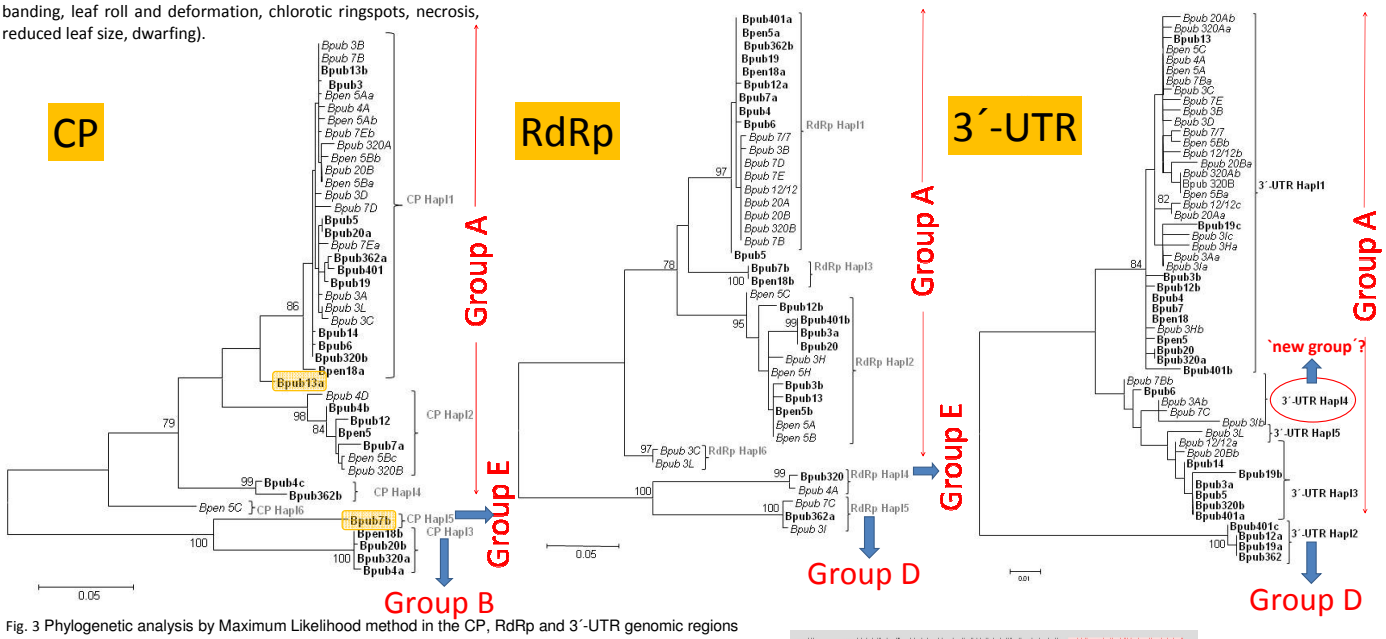

Fig. 3 Phylogenetic analysis by Maximum Likelihood method in the CP, RdRp and 3´-UTR genomic regions conducted in MEGA6 and bootstrap calculation for 1,000 replicates. The bar indicates the substitutions per nucleotide. **Bold letters**: variants from the original population; *italics*: variants from the grafted population; yellow boxes: presumably recombinants. Phylogenetic groups (groups A-E) are defined by Rebenstorf et al. (2006).

Phylogenetic analysis

❖ CLRV variants cluster into 5 - 6 well differentiated haplotypes in each genomic region (Fig. 3).

❖ One haplotype is predominant in the majority of the original and the grafted trees (Hapl1).

❖ Most haplotypes are present in both populations, there are cases where a haplotype does not „pass" to the grafted population or „new" haplotypes appear only in the grafted population.

❖ Single trees are mixed-infected by highly variable CLRV haplotypes that cluster into different phylogenetic groups (according to Rebenstorf et al., 2006).

Recombination analysis Two recombinants have been detected in the CP region:

a. CLRV variant **Bpub13a** of group A (major parent: Bpub19 - CP Hapl1; minor parent: *Bpub 4D* - CP Hapl 2) (Fig. 4A)

b. CLRV variant **Bpub7b** of group E (major parent: *Sambucus sp.* GER – Group B; minor parent: *Rheum sp.* GER – Group E) (Fig. 4B)

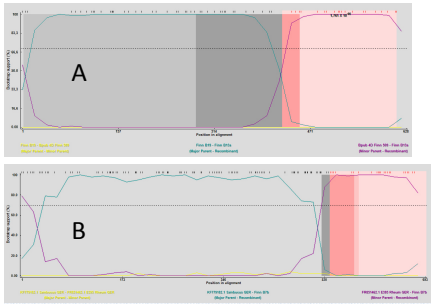

Fig. 4 Recombination detection shown by the Siscan software (incorporated in the programm RD4) in the CP region of CLRV variants **Bpub13a** (A) and **Bpub7b** (B) originating from *B. pubescens* trees.

Conclusions

✓RNA recombination: another source of genetic variation of birch CLRV population in Rovaniemi.

✓ Indication of CLRV strains from diverse hosts *Sambucus*/ *Rheum*) crossing the species barrier and successfully infecting new host followed by genetic adaptation processes.

✓Coexistence of a complex of highly variable strains in the same host: a possible factor that may have led to the disease´s emergence.

Literature Büttner, C., von Bargen S., Bandte, M. and Mühlbach, H.-P., 2013. Forests diseases caused by viruses. In: Infectious forest diseases. APS Press. ISBN: 9781780640402. Ch. 10, p. 97-110.
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