# Exploring OsHV-1 diversity at the gene and genome scale

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Preventing and mitigating farmed bivalve diseases

# Background

- Since 2008, massive mortality outbreaks in *Crassostreae gigas* oyster spat
  - Reported in several Member States
  - Attributed to the presence of the a new variant of Ostreid herpes virus-1 termed Ostreid Herpes Virus-1µVariant (OsHV-1µVar)
  - Recognised as an emerging disease in Europe & controlled under 2006/88/EC
- A better understanding of viral diversity is required to:
  - allow improved pathogen detection in hosts and in the environment
  - facilitate better control of the virus spread

# Approach

Two complementary approaches:

**1. Gene scale**: A study of the UK and Irish isolates collected since 2009

- focused on 2 variable regions the C2/C6 and ORF 49/50
- establish the genetic relationship between the outbreaks in the UK
- establish if there has been spread of OsHV-1 within the designated compartments/buffer zones in the UK.
- Investigate the genetic relationship between the Irish sequences & between the UK and Ireland sequences.

**2. Genome scale**: A study of isolates from different areas and time points

- To gain a better understanding of the development of the virus in time and space.

# Gene Scale Study: UK & Ireland Outbreaks

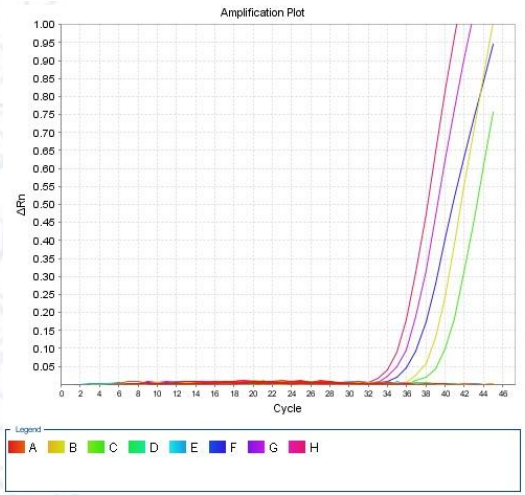
United Kingdom:

- first OsHV-1 µvar outbreak was reported in UK in 2010 in Whitstable during the first year of the Surveillance Programme for the early detection of OsHV-1 µVar
- Further outbreaks of OsHV-1 µVar occurred in the UK in 2012, 2013 and 2015 in Maldon, Essex and Poole Harbour, Dorset and River Teign, Devon respectively
- Maldon and the River Teign: Sequences identical to the OsHV-1µVar sequence from Whitstable, whereas, the presence of a G insertion in the C2-C6 region was identified in the Poole Harbour samples from 2013
- 3 other sites infected with OsHV-1 “wild-type” – missing the characteristic deletion in the C2-C6 region

Ireland:

- First mortality associated with OsHV-1 µVar was first recorded in 3 bays (Woodstown Strand, Dungarvan and Castlemaine Harbour) in 2008.
- In 2009, outbreaks were again recorded in these sites as well as a further 14 bays around the coast following spat imports from France
- 2010: Surveillance programme initiated in those areas remaining free
- 2010-2018: Virus continues to spread
- Previous study sequencing of the C2-C6 region of isolates collected between 2008 & 2012 showed presence / absence of G insertion described in the UK

# Locations of the OsHV-1 µVar outbreaks in the UK and the detection of a wild-type virus




## Methodology

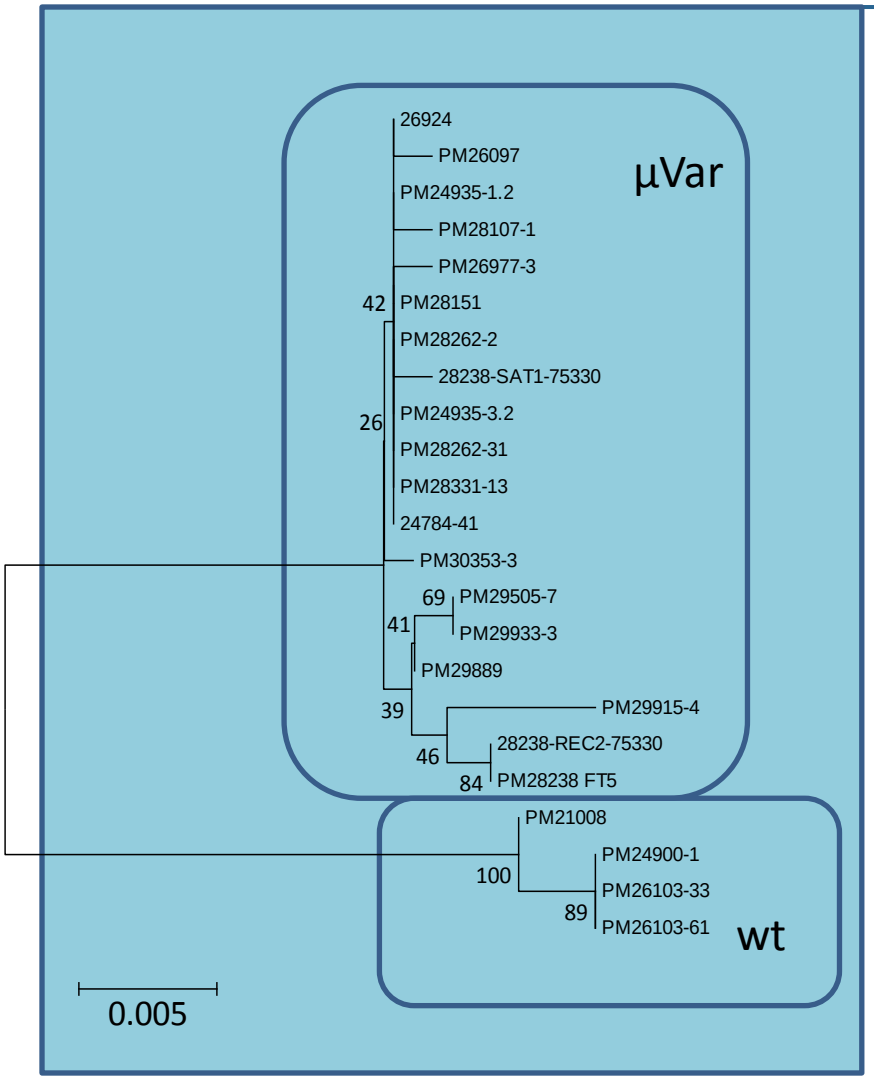
**DNA Extraction:** Qiagen DNA Mini kit

**Conventional PCR** using GoTaq (Promega, UK) :

1. Confirmation of OsHV-1 µVar : C2/C6 primers (Arzul et al 2001) with internal primers OsHV-1 fint and OsHV-1 rint (Stone, unpublished)
2. Subsequent virus characterisation: Hypervariable region in ORF49/50 - ORF49/50 IGR F & R, ORF49/50 IGR Fint & Rint (Stone, unpublished)

**Sequencing**:

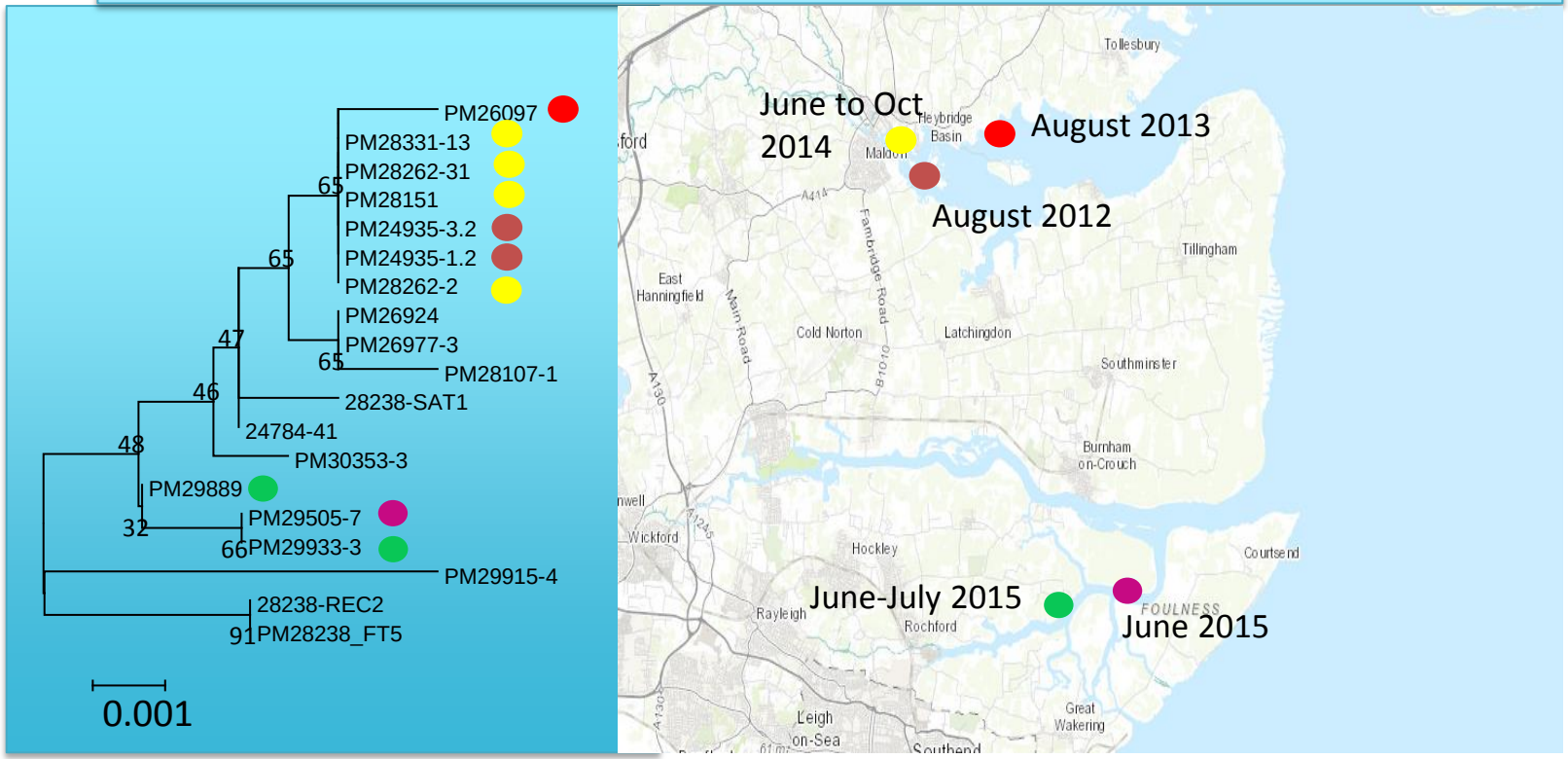
Sequencing Rx: ABI PRISM Big Dye Terminator v3.1 cycle sequencing kit analysis: 3500xl genetic analyser; consensus sequences generated using CLC workbench software (Qiagen); multiple alignments were performed using Clustal W (Thompson et al. 1997) with the following Clustal parameters: a gap opening penalty of 15 and gap extension penalty of 6.66. Phylogenetic analyses were conducted using MEGA version 4 (Tamura et al. 2007); neighbour-joining tree was constructed using a maximum composite likelihood model, and the robustness of the tree was tested using 1000 bootstrap replicates.



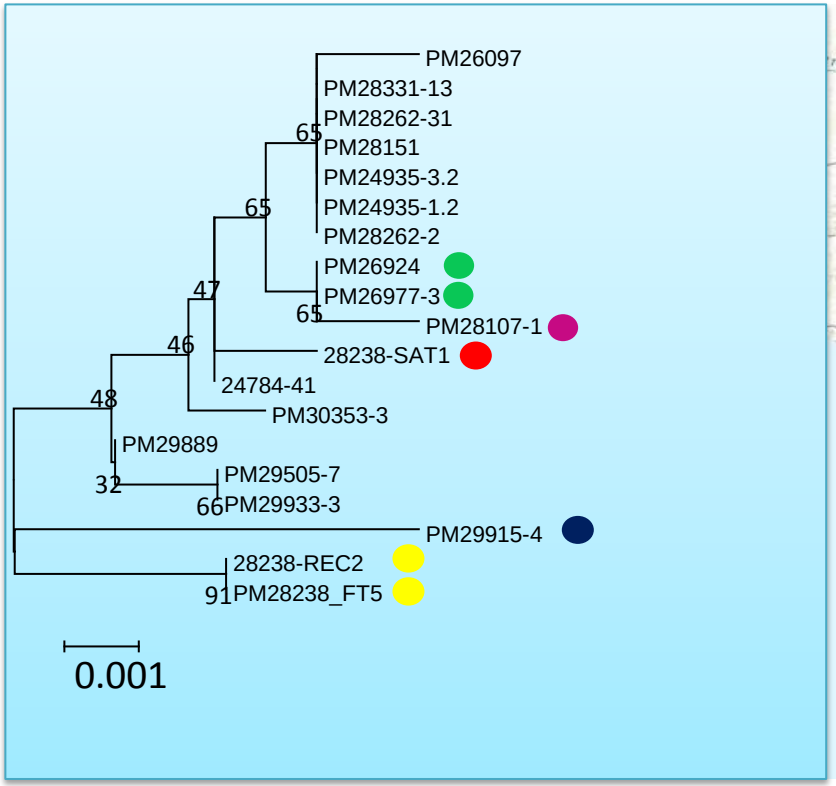

# Results UK

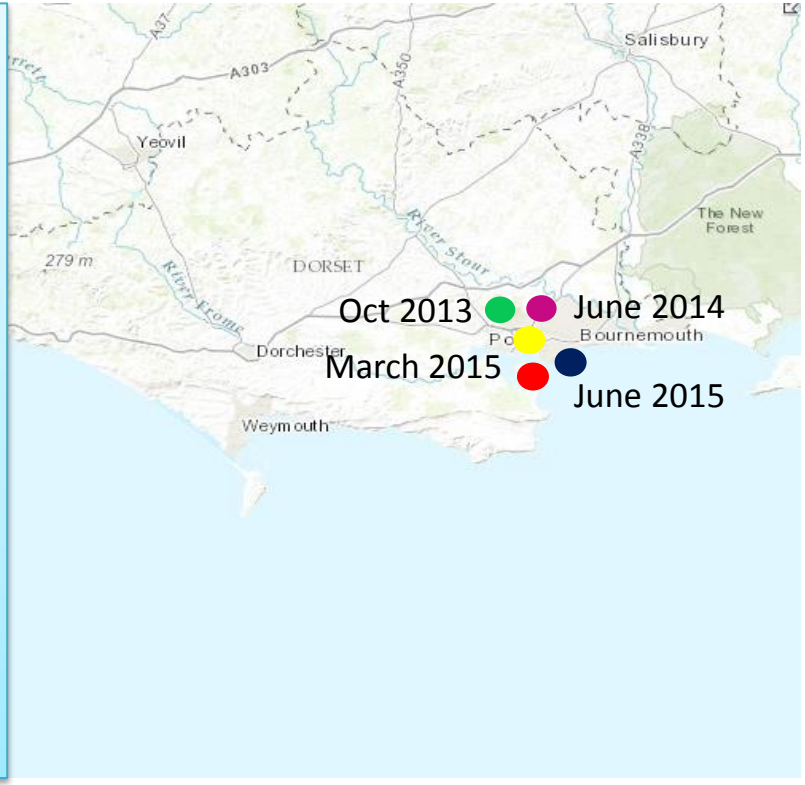
- Sequence Divergence was low
- Nucleotide substitutions and deletions that are shared between some sequences suggesting the OsHV-1 in the samples represent viruses from the same independent sources
- Initial analysis: 2 lineages Wild Type and µVar
  Wild type found in: River Blackwater, the River Colne, River Crouch and the River Roach
- Reanalysis of the OsHV-1 µVar samples provided greater resolution and the separation of isolates into multiple sub lineages which potentially represent viruses originating from distinct geographical sources.
- Following the initial outbreak on the Whitstable site in 2010 all subsequent detections on the site share 100% nucleotide identity – remaining stable over an 8 year period

# Characterisation of the viruses associated with a spread of OsHV-1 in the Maldon, River Coln and River Crouch

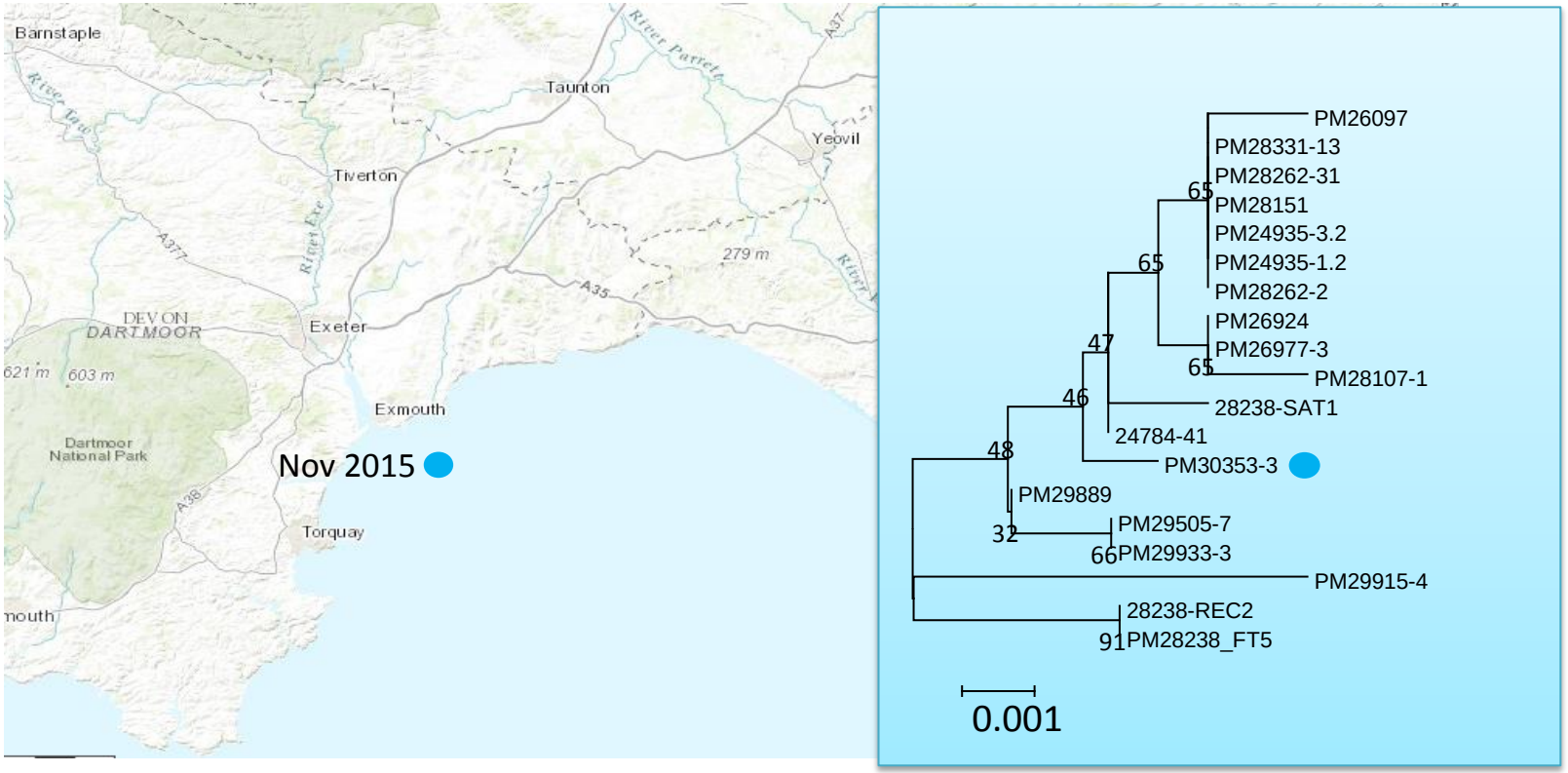


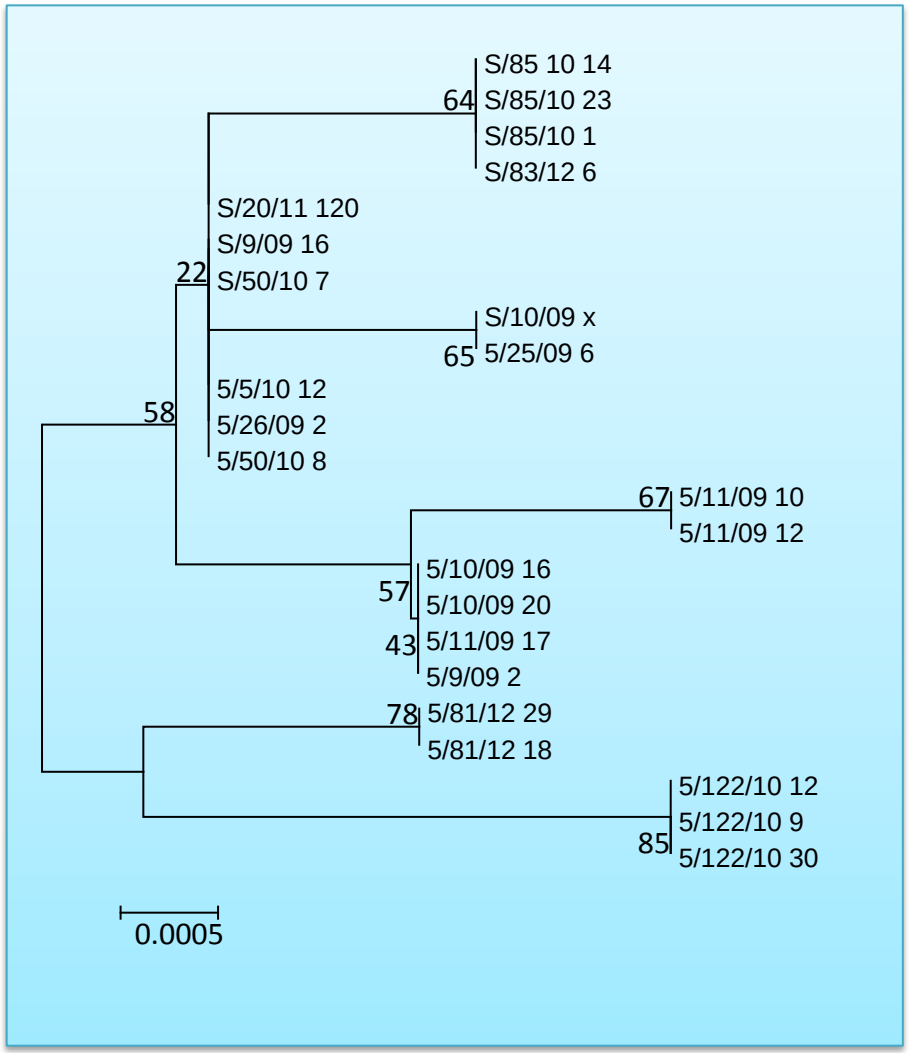
# Characterisation of the viruses associated with the continued infection OsHV-1 in Poole Harbour



# New outbreak of OsHV-1 on the River Teign in 2015






# Results Ireland

- More open trade with France, multiple onputs from France each years
- As with UK, Ire sequences share a high degree of similarity
- All sequences obtained were from the OsHV-1µVar lineage
- Multiple substitutions, deletions and insertions evident
- A no. of substitutions, insertions and deletions are shared between sequences collected in different bays at different time points - shared stock source / trade links between bays?
- different sequences are observed over time within the same bay as well within the same outbreak confirming the likelihood of multiple introductions each year as well as between years
- Samples were included where mortality patterns had differed from other outbreaks: sequences within the an outbreak identical or closely related but differed from other isolations – relevance?

# Comparison of UK and ROI sequences

- Clear differences and similarities were evident between sequences in the ORF49/50 region
- Notable finding included:
    - 2 sequences from an outbreak in Carlingford in 2011 were identical to sequences from the 2010 Whitsable outbreak
    - Three ROI  samples; two from 2012 and one from 2015 from Woodstown Strand shared 100% nucleotide identity with a sample from the R.Teign from 2015 in both the C2-C6 and ORF49/50 regions,
    - Several samples from the RoI from 2009 from Carlingford & Dungarvan shared 100% nucleotide identity to a Poole Harbour samples from 2013 in the ORF 49/50 region with a single C to G substitution, but did not have the additional G observed in the C2-C6 region

# Conclusions

- 4 distinct geographical areas of the UK are positive for OsHV-1
- Each represents a new outbreak that is genetically distinct  from the outbreaks
- Increased testing have revealed additional OsHV-1 variants n the same site -  new introductions or mixed infections
- The picture which emerges from Ireland is suggestive of multiple introductions over time as would be expected where the industry relies on importation of spat annually from France.
- There are commonalities between isolates which would point to the same isolates being introduced into multiple sites from the same source although trade links between bays within Ireland may also be responsible for some of these disseminations.
- A comparison of the UK and Irish isolates suggests connections which would point to the source being the same in both countries as no trade exists between the impacted Irish and the UK sites

# Genome Scale Study: Isolates from different areas and time points

- To gain a better understanding of the evolution and the diversity of the virus in time and space.

Hypothesis : DNA virus are not stable

1- Different structural variants should be found in different host species.

2- Distinct OsHV-1 variants should be found within one infected individual.

# Biological materials

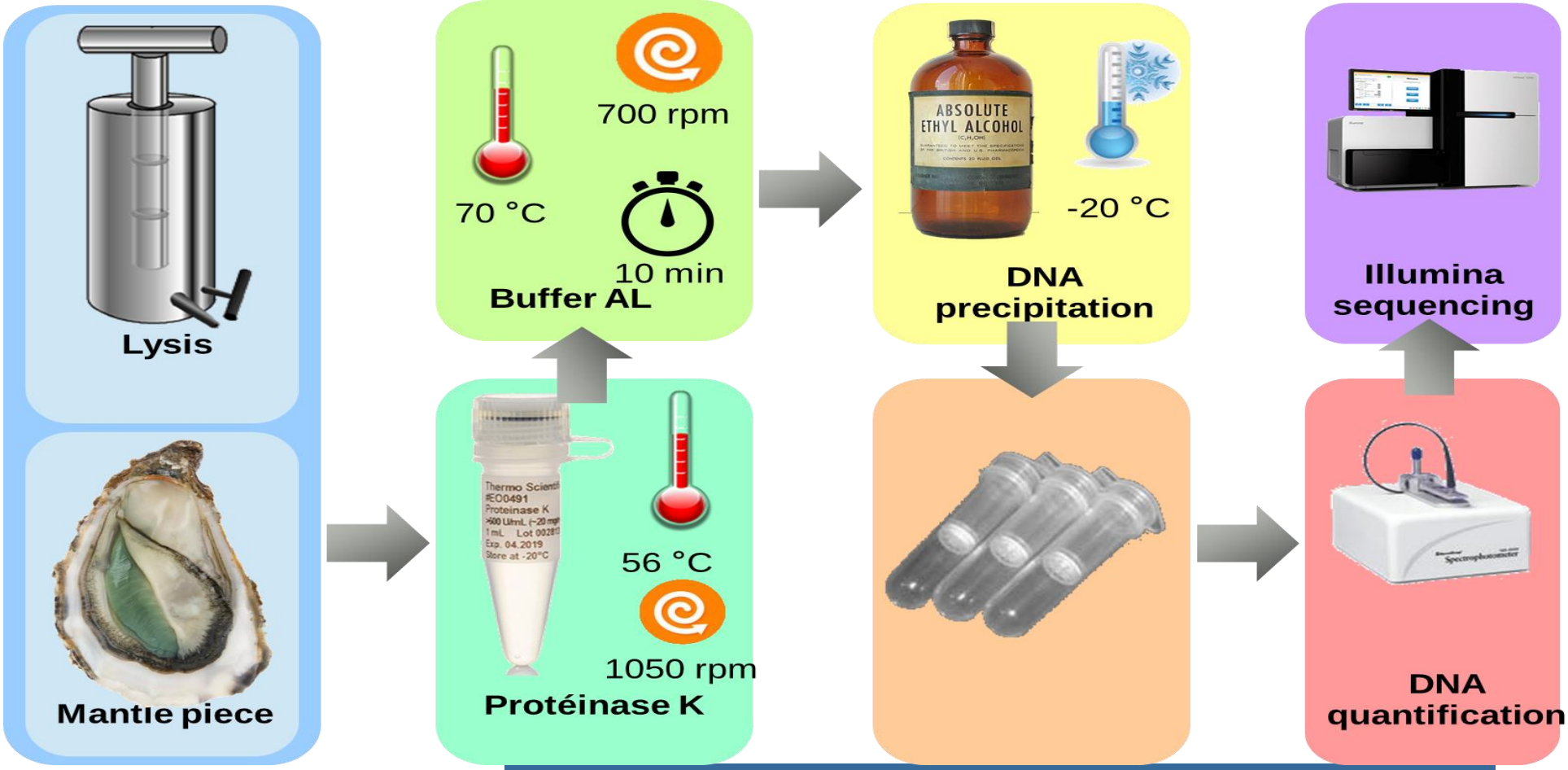
| | | | | |
|---|---|---|---|---|
| Poole-Harbour | UNITED-KINGDOM | | 2015 | adult |
| VIV | FRANCE | | 2017 | 7 Adults |
| LI | | | 2010 | Pool of larvae |
| PR | | | 2008 | Pool of juveniles |
| MV | | | 2010 | Ripe Adult |
| NZ | NEW-ZEALAND | C. gigas | 2010 | Pool of larvae |
| NZ16 | | | 2011 | adult |
| NZ17 | | | 2011 | adult |
| NZ18 | | | 2011 | adult |
| SW6 | SWEDEN | O. edulis | 2012 | adult |

Samples from the WP3

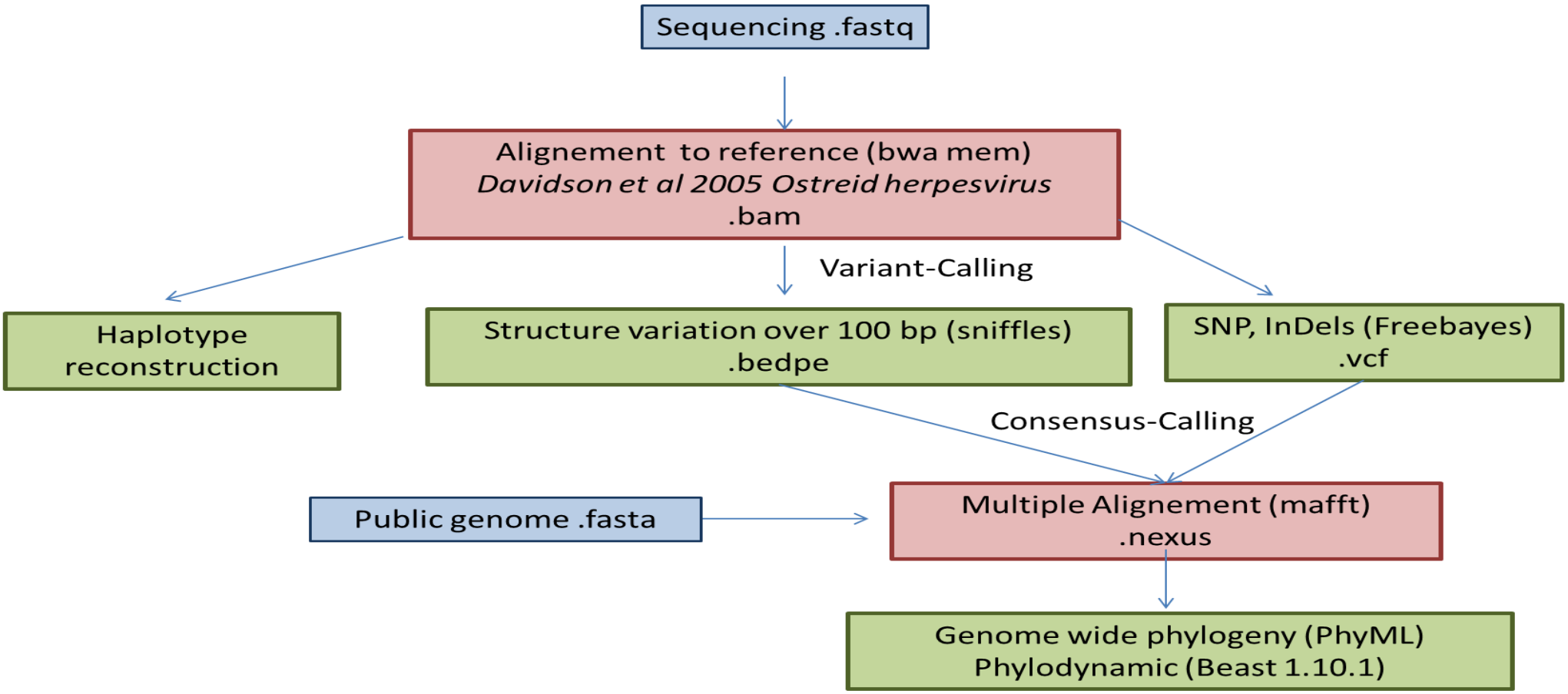
Two species : *Crassostrea gigas* and *Ostrea edulis*

Time span : 2008-2017

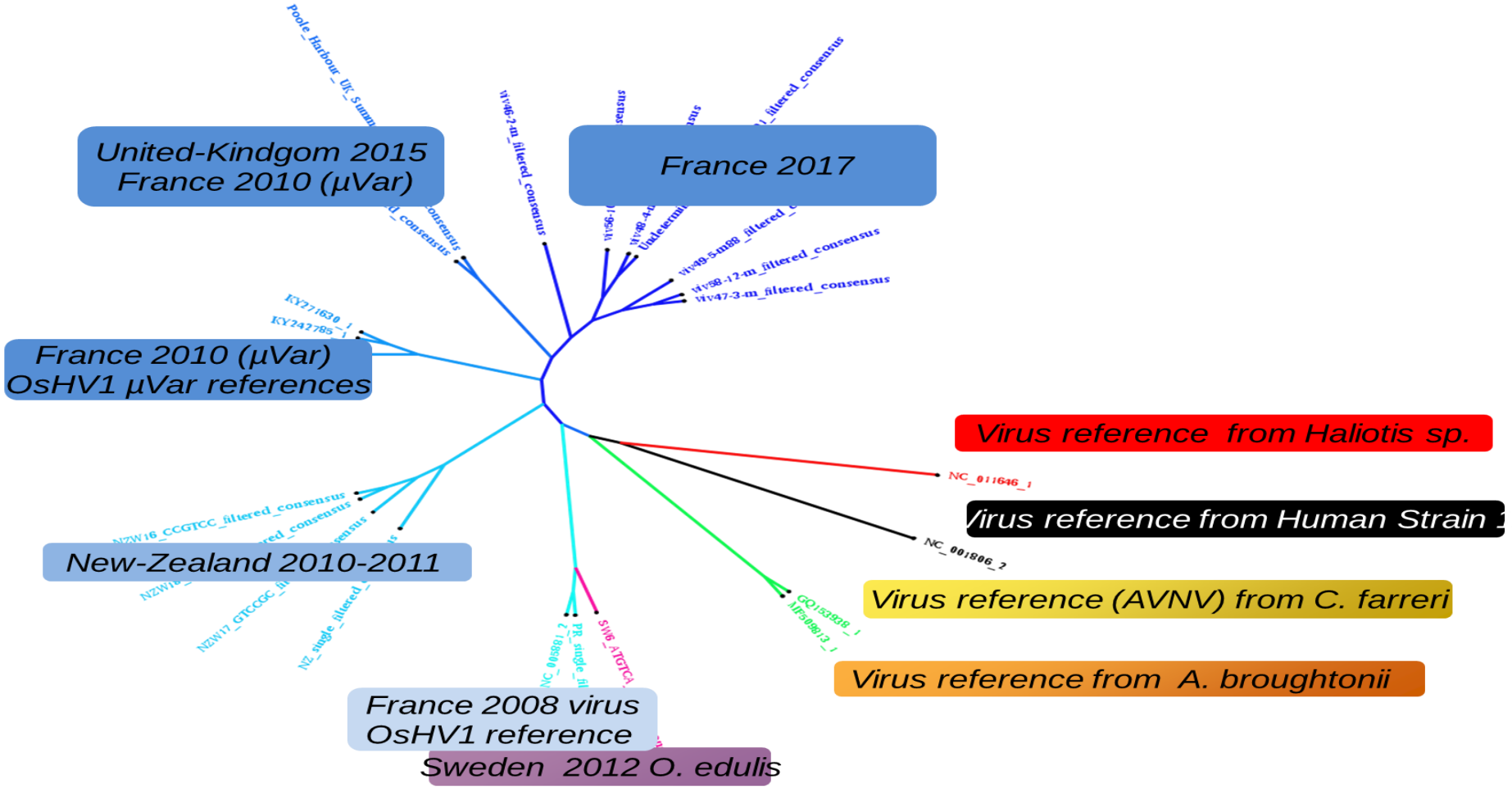
# Conventional to Ultra-deep sequencing



# Bioinformatic pipeline

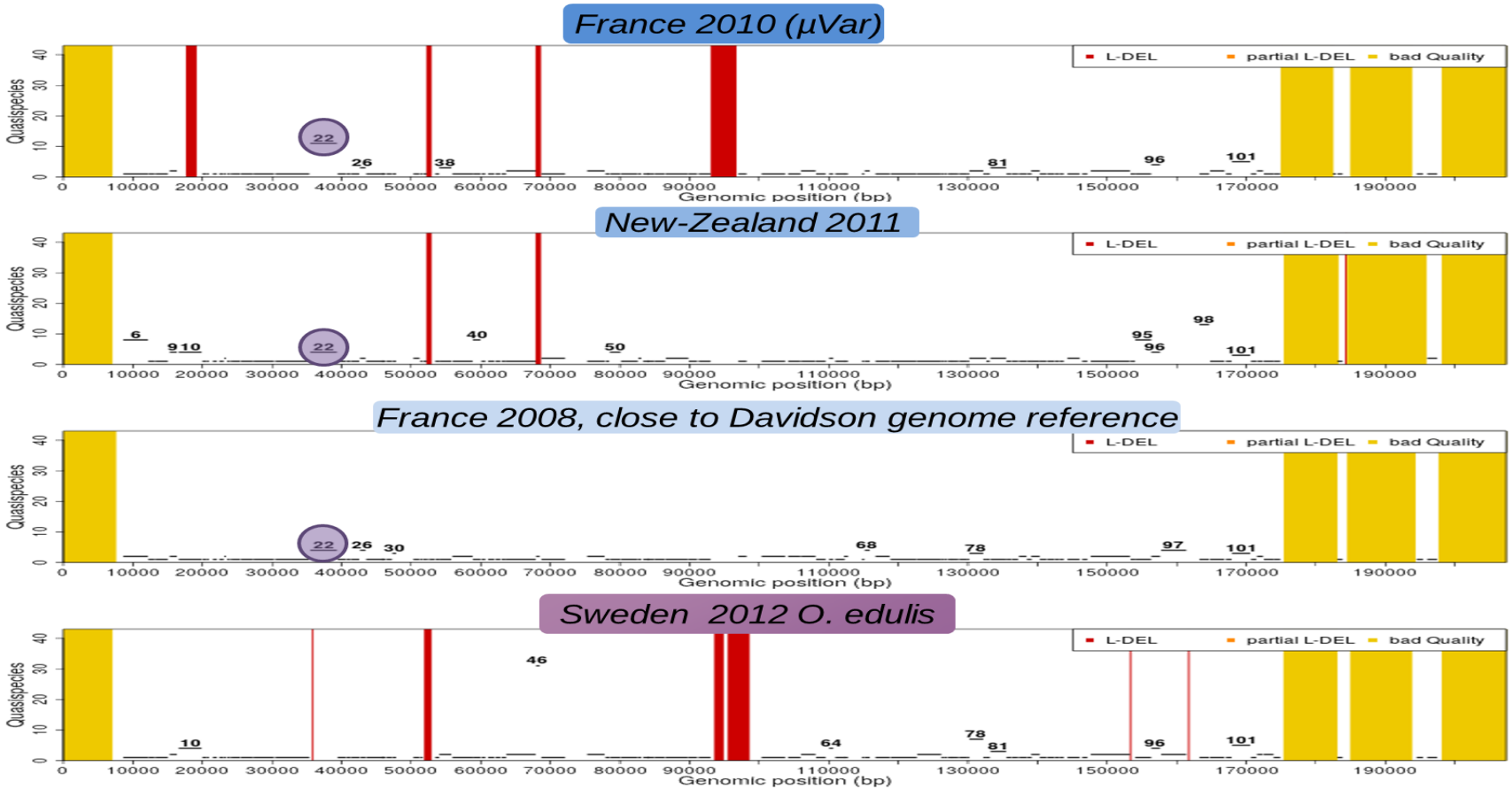


# Genome-wide phylogenetics



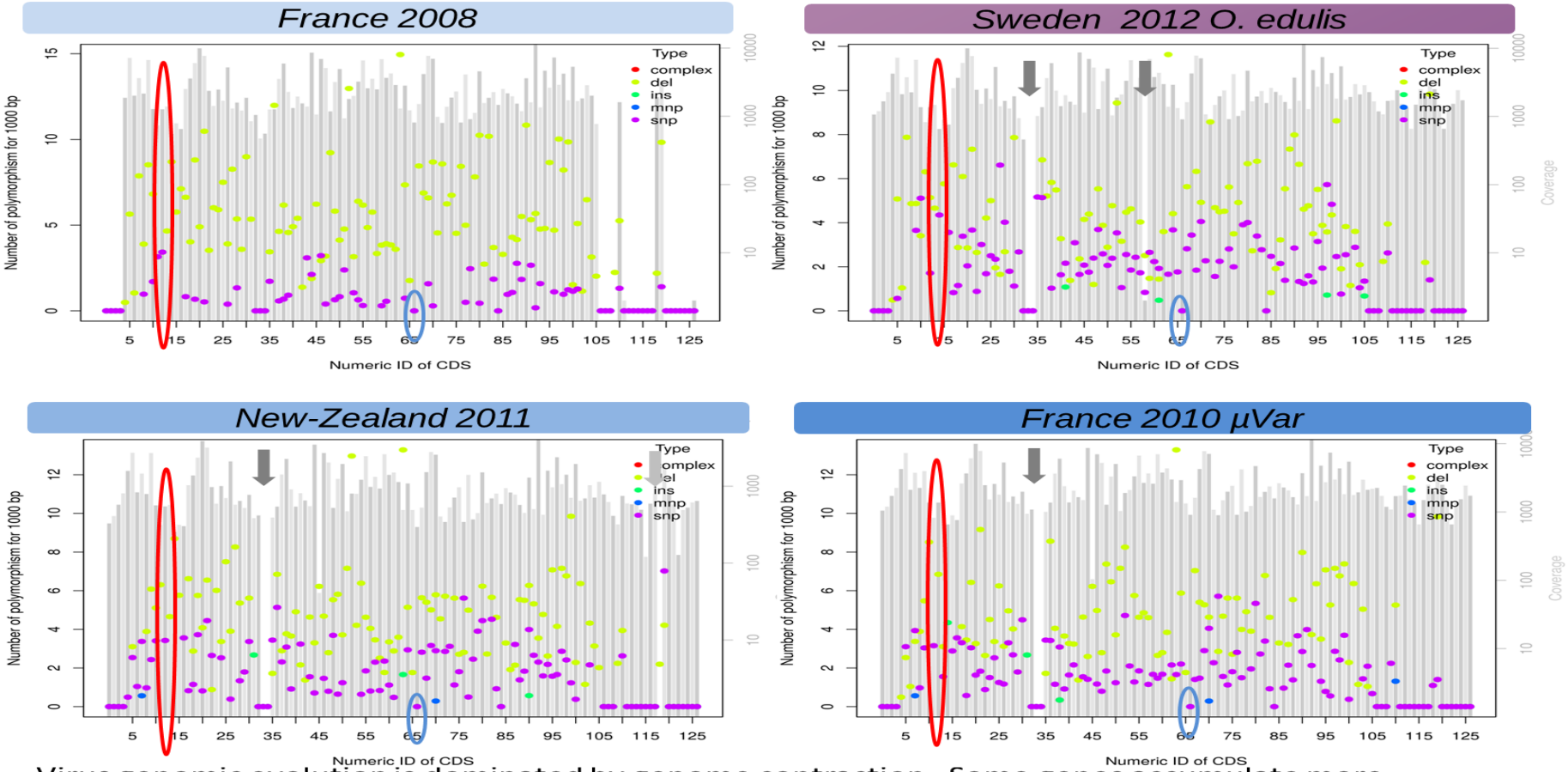

Lamy and Morga in prep

# Structural variants and Haplotype diversity




Lamy and Morga in prep

# SNP, Indels per genes




Virus genomic evolution is dominated by genome contraction. Some genes accumulate more substitutions (*Davision et al 2005 as reference*) compare to other genomic regions.

# Take home message

1- There is not sharing of viral haplotype between host. On each host, viral haplotype exhibits large structural variation.

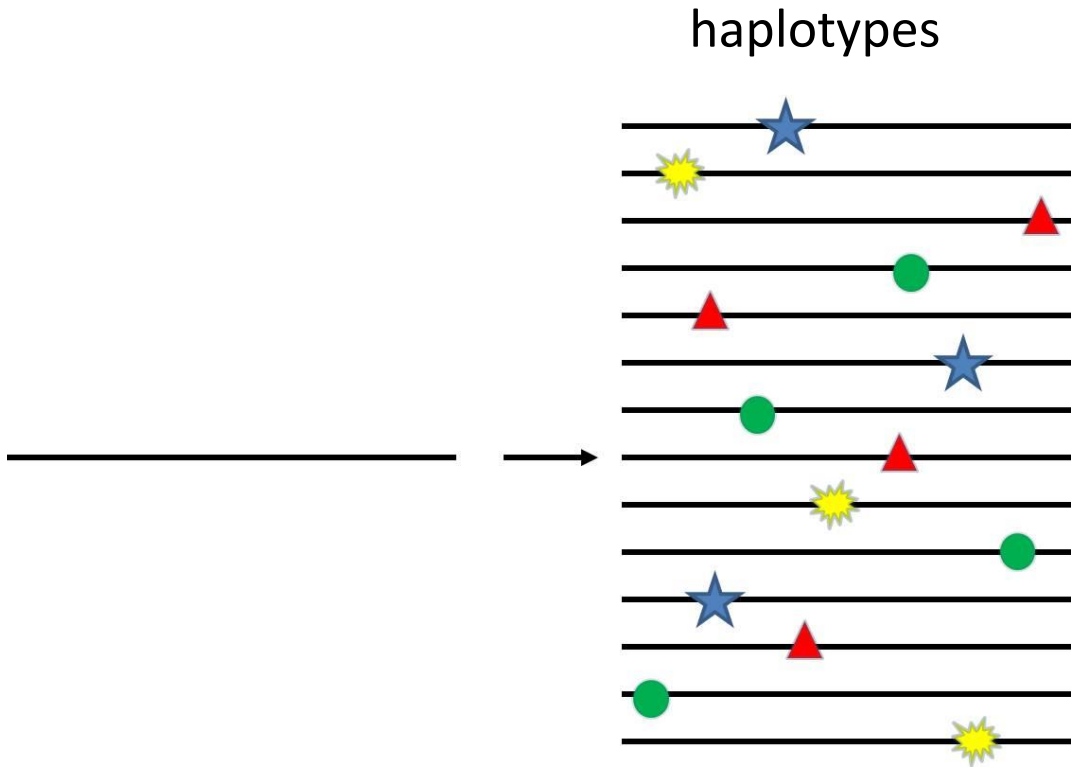
2- In one infected individual there is (at least) between 2 and 20 related viral haplotypes.

3-Indeed, OsHV-1 µVar is not an homogenous group… OsHV-1 µVar is a swarm of (related) structural variants. Quite different haplotype variants have been called *"µVar".*

4- It seems that OsHV-1 is able to shift from host to host quickly.

5- Large Deletions and substitutions are not random in the genome, it means that genetic drift and natural selection (though host immunity ?) are acting on viral populations.

# Take home message




Dr. Jean-Baptiste Lamy

# Conclusions

1- Two complementary studies were carried out to investigate the diversity of OsHV-1 with a view to gaining a better understanding of the diversity of the virus.

2- The first study focused on two regions of the viral genome in samples collected from outbreaks in the UK and Ireland. The results indicate that the four outbreaks in the UK are most likely the result of separate introductions. The results from Ireland are suggestive of multiple introductions both over time but also within the same bay in the same year.

3- There are also commonalities between isolates from Ireland and the UK suggestive of common sources for these outbreaks

4-The second study, based on a whole genome sequence approach, included samples from different parts of the world, collected at different points and used reference mapping in order to assess the viral evolution since 2005. The study shows that OsHV-1 diversity is structured firstly by host species, then by origin and finally by time.

5- The work on diversity will also contribute to better disease control in other ways. Tools targeting the more conserved regions will ensure a better detection of strains as the virus continues to evolve. The study at the genome level that OsHV-1 has incorporated a large number of mutations since its first description, however, additional work is needed to fully understand how the OsHV-1 genome structure is conserved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