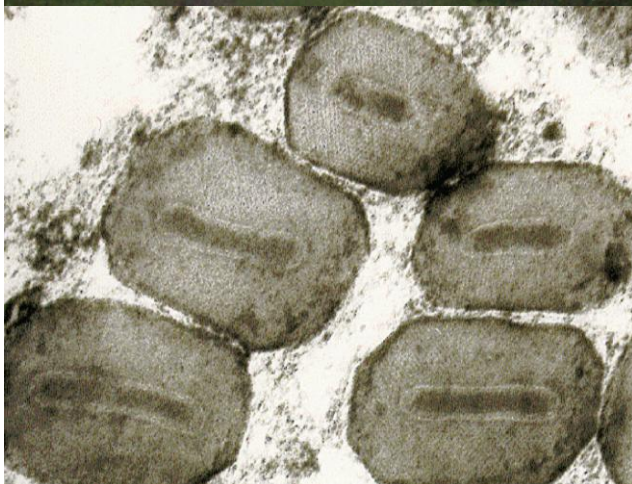
Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Specificity of insecticidal Baculovirus Strains



J. A. Jehle

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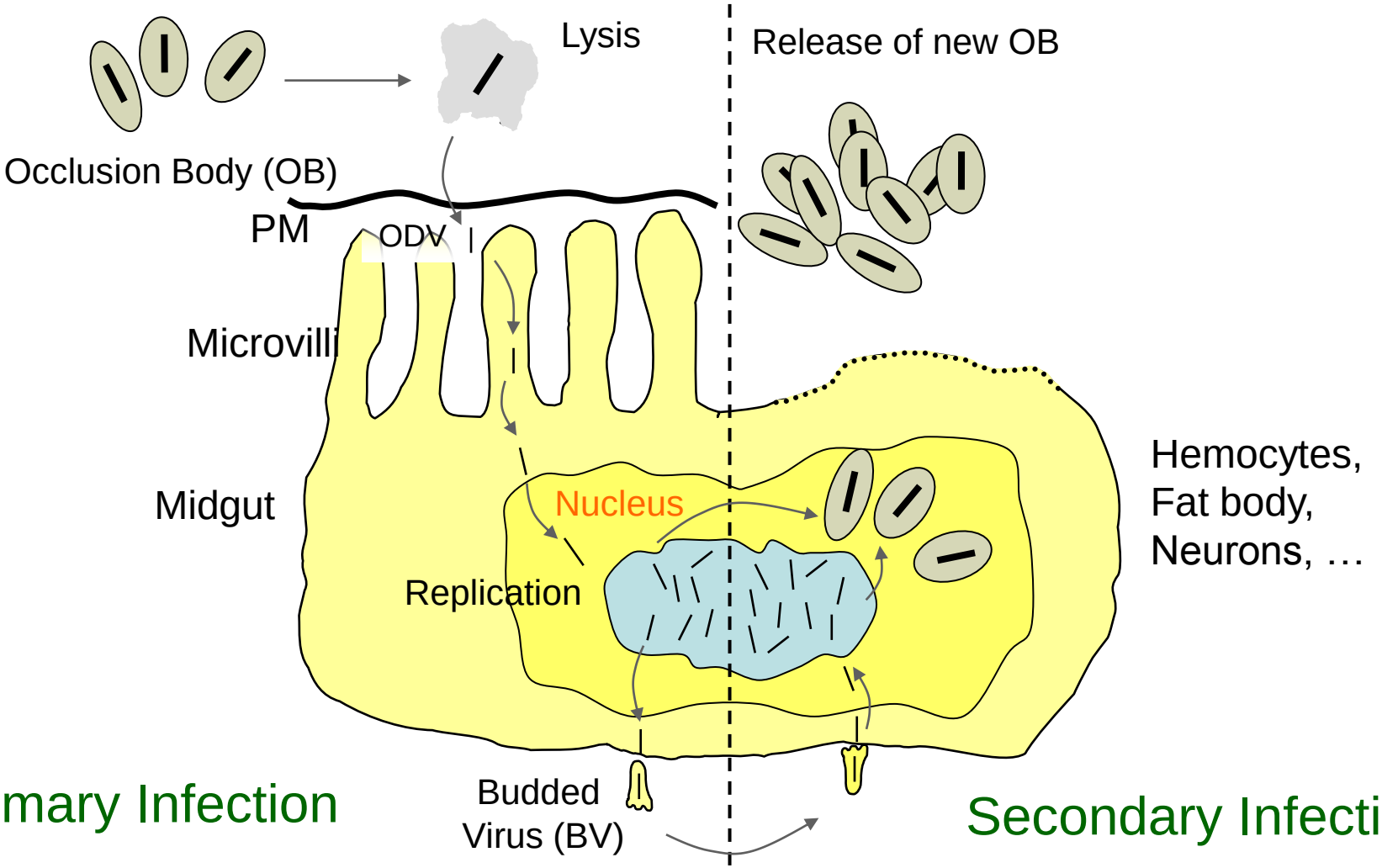
Characteristics of Baculoviruses

- rod shaped virions embedded in an occlusion body
- from more than 700 insect species isolated
- > 120 Baculovirus genomes fully sequenced
- dsDNA genome, 80 - 180 kb, 100 - 150 genes
- 38 core genes shared by all baculoviruses
- specific for Lepidoptera, Diptera, Hymenoptera

Baculovirus infection cycle



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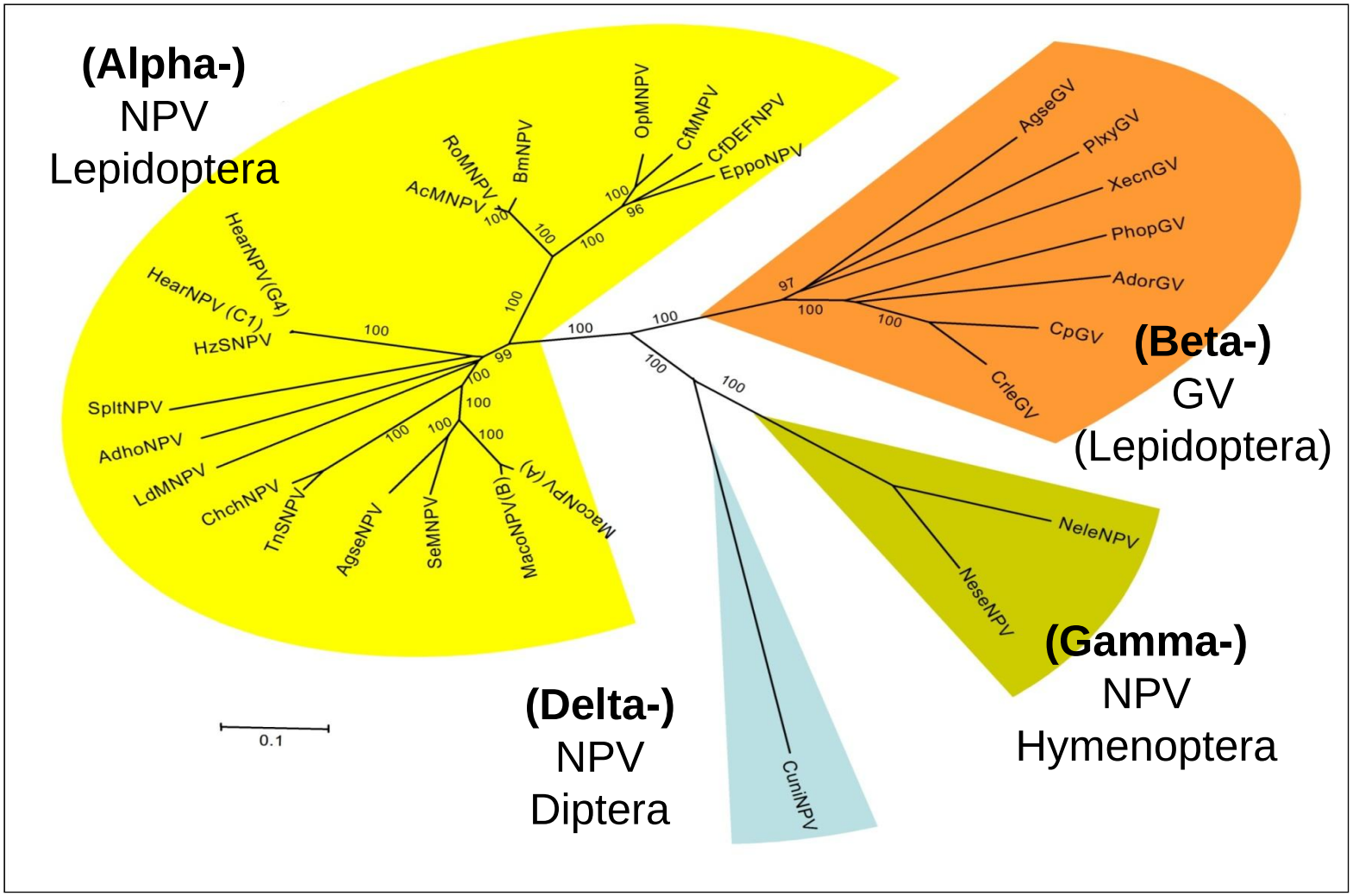
Classification of Baculoviruses



Baculovirus Occlusion Bodies

- OBs consist of multiple molecules of one protein (Polyhedrin/Granulin) in a paracrystaline structure
- GV OB are small and highly regular protein crystals
- OBs serve as a natural formulation, can be applied with conventional sprayers
- Mixed with fungicides and others, if pH < 9

Baculoviruses in Insect Pest Control

Virus	Use
Anticarsia gemmatalis MNPV	Brasil: ~ 0.3 (1-2) Mio ha
Heliothis armigera NPV	China: ~ 200 000 ha
Spodoptera exigua MNPV	Europe/US: greenhouse
Neodiprion sp. NPV	Canada
Spodoptera littoralis NPV	several countries
Cydia pomonella GV	Worldwide: > 200 000 ha
Adoxophyes orana GV	Europe: several 1000 ha
~ 15 other baculoviruses	

Authorization of Biological Control Products in Germany/EU

Microorganisms/ Viruses	Semiochemicals Pheromons	Natural Substances Botanicals

Approval of a.i. and authorization of PPP according to **(EC) Nr. 1107/2009** and **Plant Protection Law**

Some Baculovirus Specifics

- OECD Consensus document No. 20 (2002):
  „baculoviruses are safe for humans, animals and environment“
- REBECA Proposal to **approve baculovirus active ingedient on species level** (2006)

  Realized in SANCO/0253/2008 rev. 2. (2008)

  CONSEQUENCE: fast registration of isolates of the same species

Qualified Presumption of Safety (QPS) status (EFSA BIOHAZ panel)

Referring to QPS status, baculoviruses are considered as **low risk on family level** (Reg. (EU) 2017/1432)

Cydia pomonella granulovirus (CpGV)

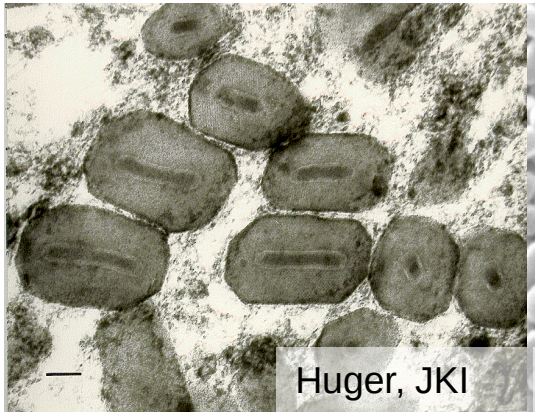
- first isolated in 1963 (CpGV-M) in Mexico (Tanada 1964)
- Important characteristics:

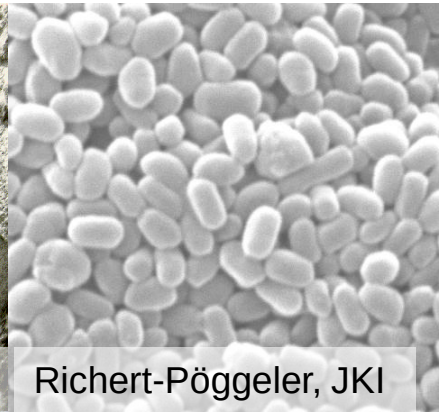
  very narrow host range

  five tortricid species recorded as susceptible

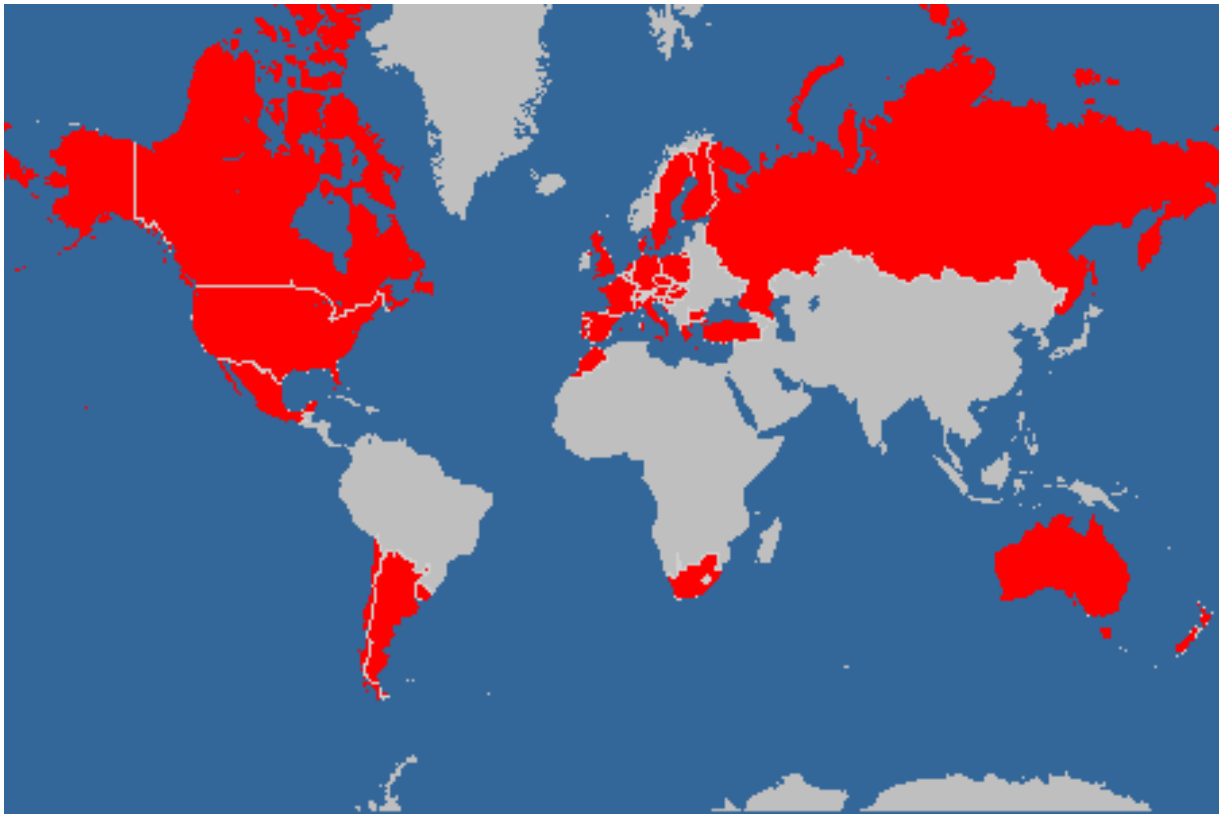
  highly virulent for codling moth larvae

  fast killing GV (5-7 days)
- Type species of *Betabaculovirus* genus





CpGV: Registered and used in 34 countries world-wide



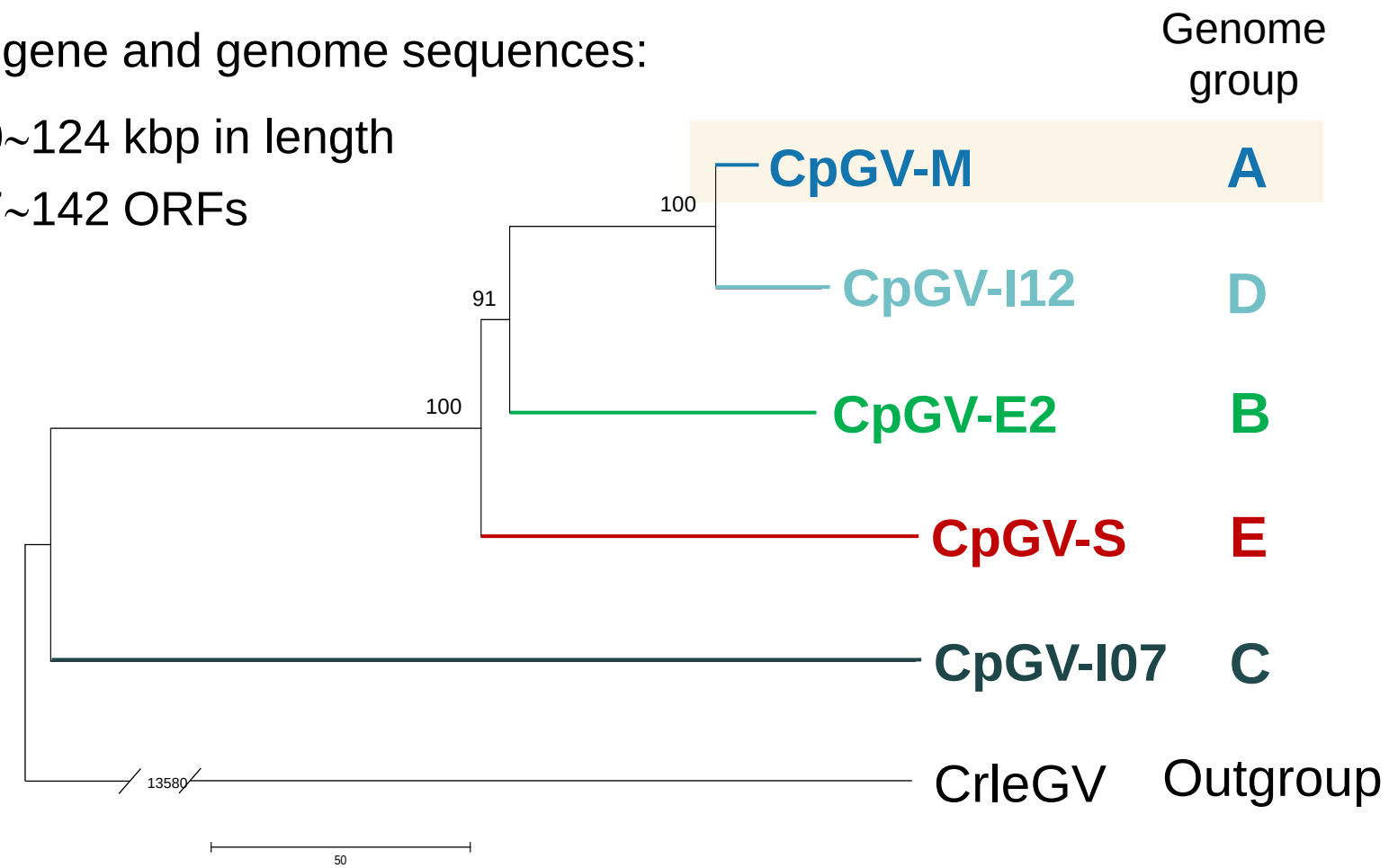
http://douweosinga.com/projects

Europe:	23
America:	6
Africa:	2
Asia:	1
Australia and Pacific:	2

Phylogeny of CpGV Isolates

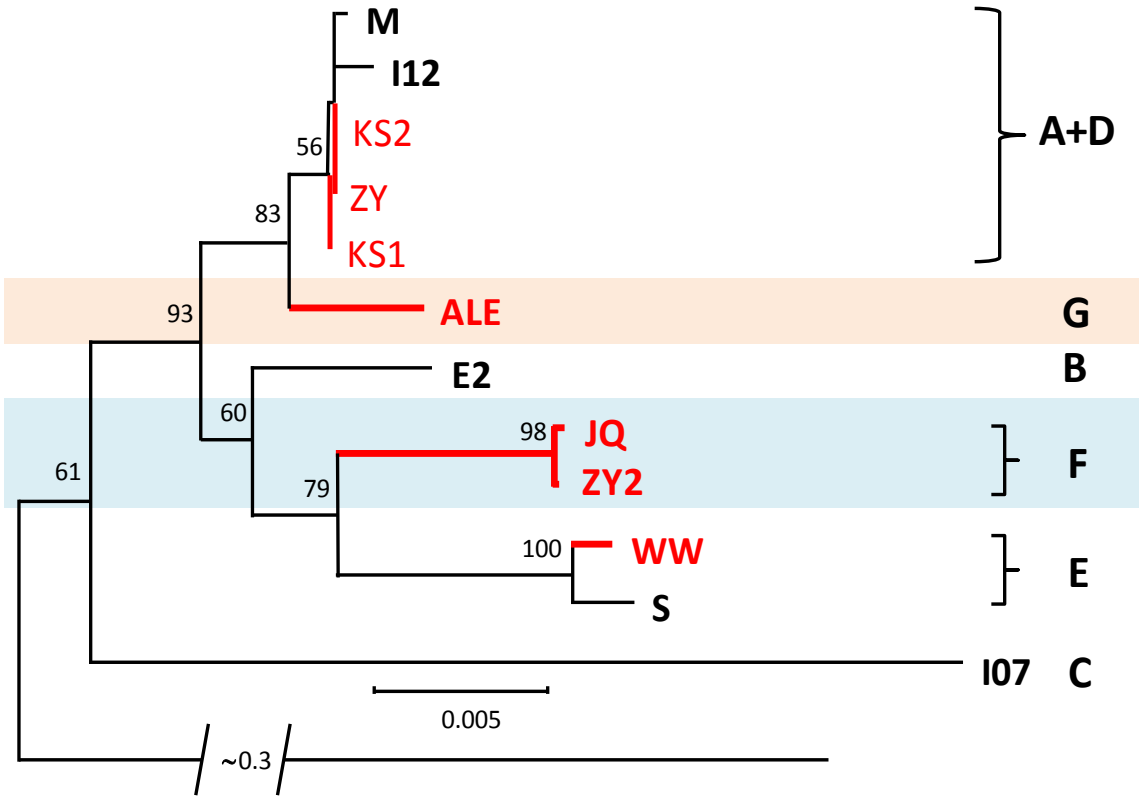
Single gene and genome sequences:

- 120~124 kbp in length
- 137~142 ORFs

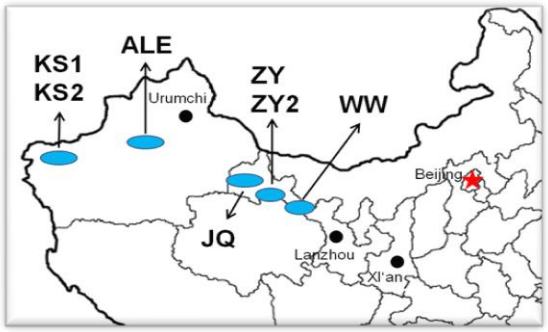


Eberle et al. JGV 2009; Gebhardt et al. PNAS 2014; Wennmann *et al.* (2017) Viruses

Increased genetic diversity in new CpGV isolates



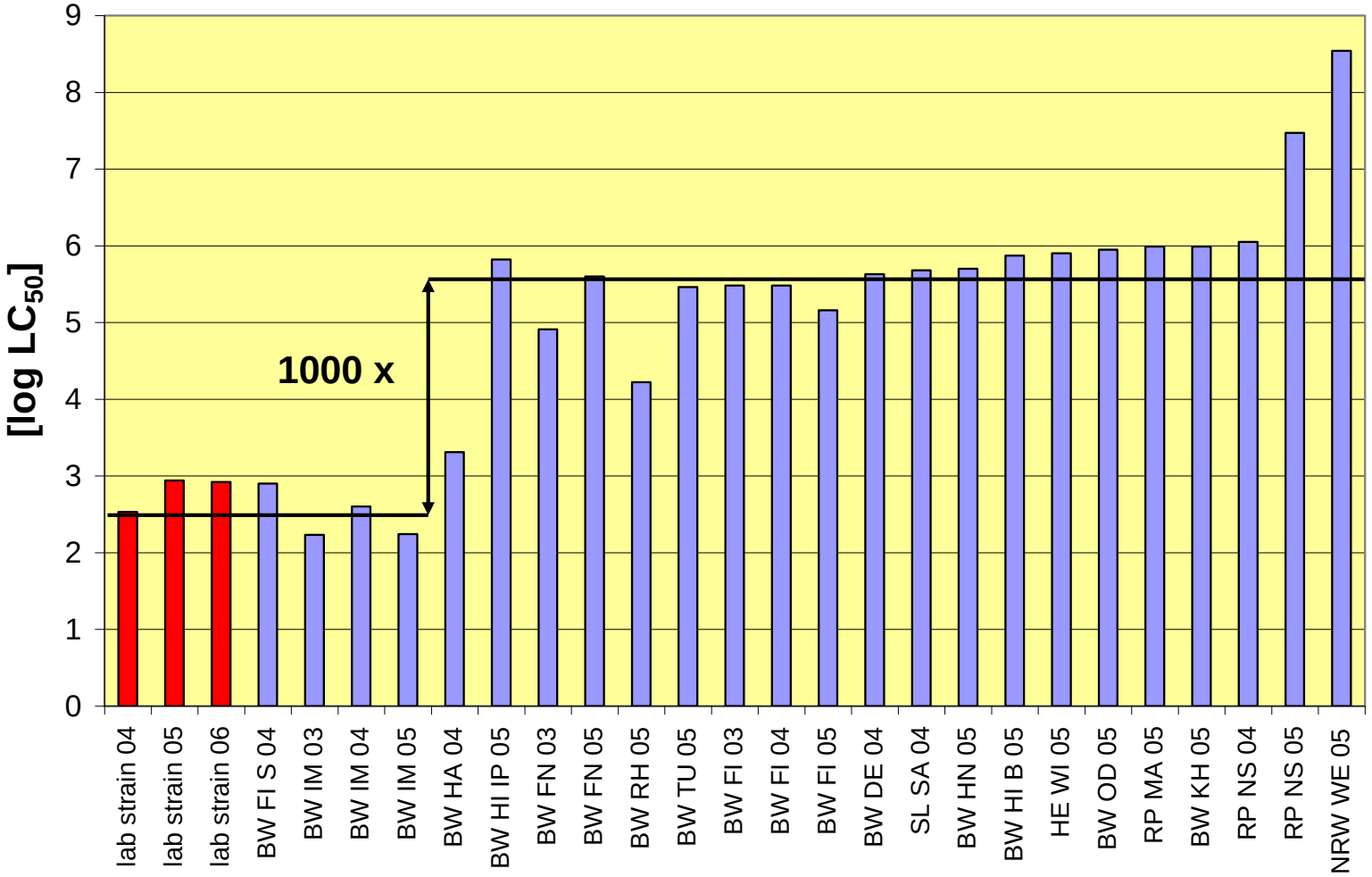
Fan et al. 2020 (Virology 541, 32-40)



- Two new phylogenetic lines, group F and G
- Not all isolates follow current picture of resistance breaking

CpGV-SA: Motsoeneng et al. Viruses 2019

Emergence of resistance to CpGV in Germany

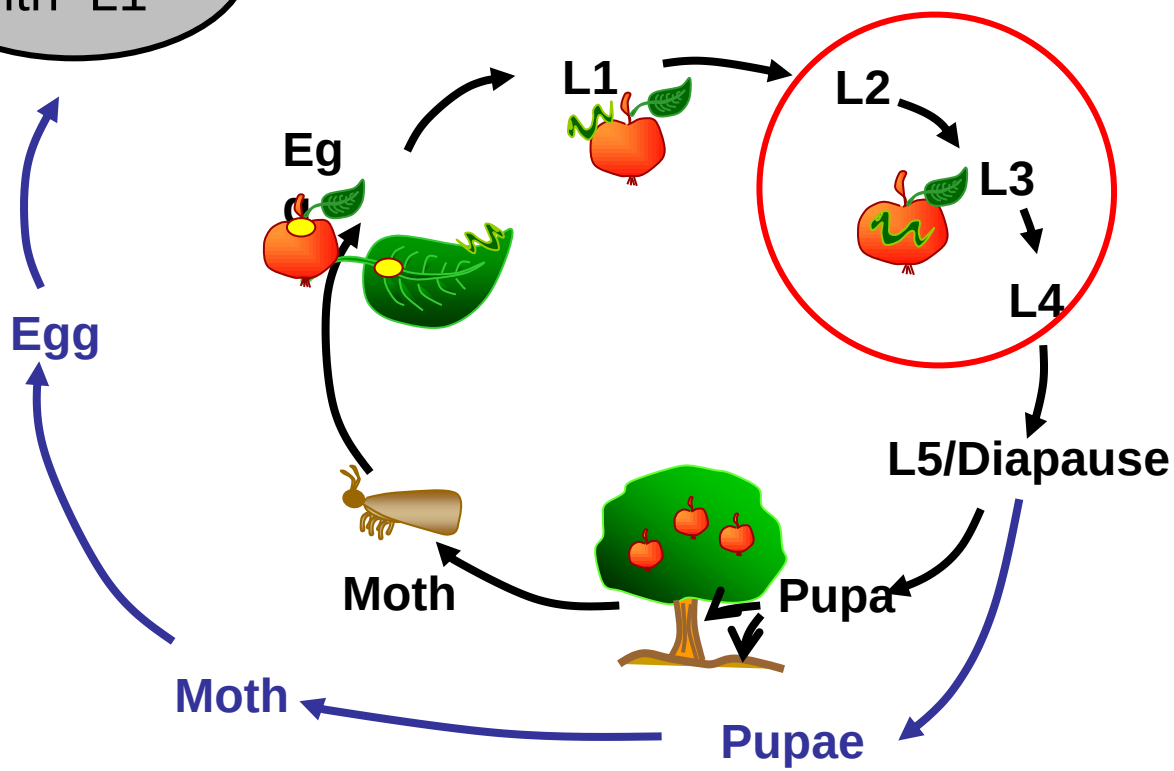


Asser-Kaiser et al. SCIENCE 2007

Resistance Testing with CpGV

Bioassay with L1

Collect L2-L4, Laboratory quick test

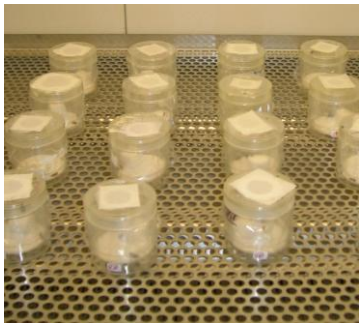


103 field samples:

> 23 500 larvae

Collect diapausing larvae in autumn
Rearing the population in the lab

Inheritance of CpGV Resistance: Experimental Approach



Single pair crossing

Testing larvae on virus

Testing larvae on virus

Resistant line

Mass crossing

Rearing larvae on virus

Rearing larvae on virus

Resistant line

Backcrossing with susceptible line

Three Types of CpGV Resistance

Field Population	Selected Strain	Inheritance Type	Resistance Type	References
CpR	CpRR1	**Z-linked** Dominant **Monogenic**	**Type I**	Asser-Kaiser et al. Science 2007
NRW-WE	CpR5M/ CpR5S	**Autosomal** Dominant **Monogenic**	**Type II**	Jehle et al. AEM 2017; Sauer et al. PLOSone 2017
SA-GO	CpRGO	**Autosomal/ Z-linked**, Dominant **Polygenic**	**Type III**	Sauer et al. AEM 2017

Occurrence of CM Populations resistant to CpGV

- Demonstrated resistance
- CpR = Type I
- NRW-WE = Type II
- SA-GO = Type III

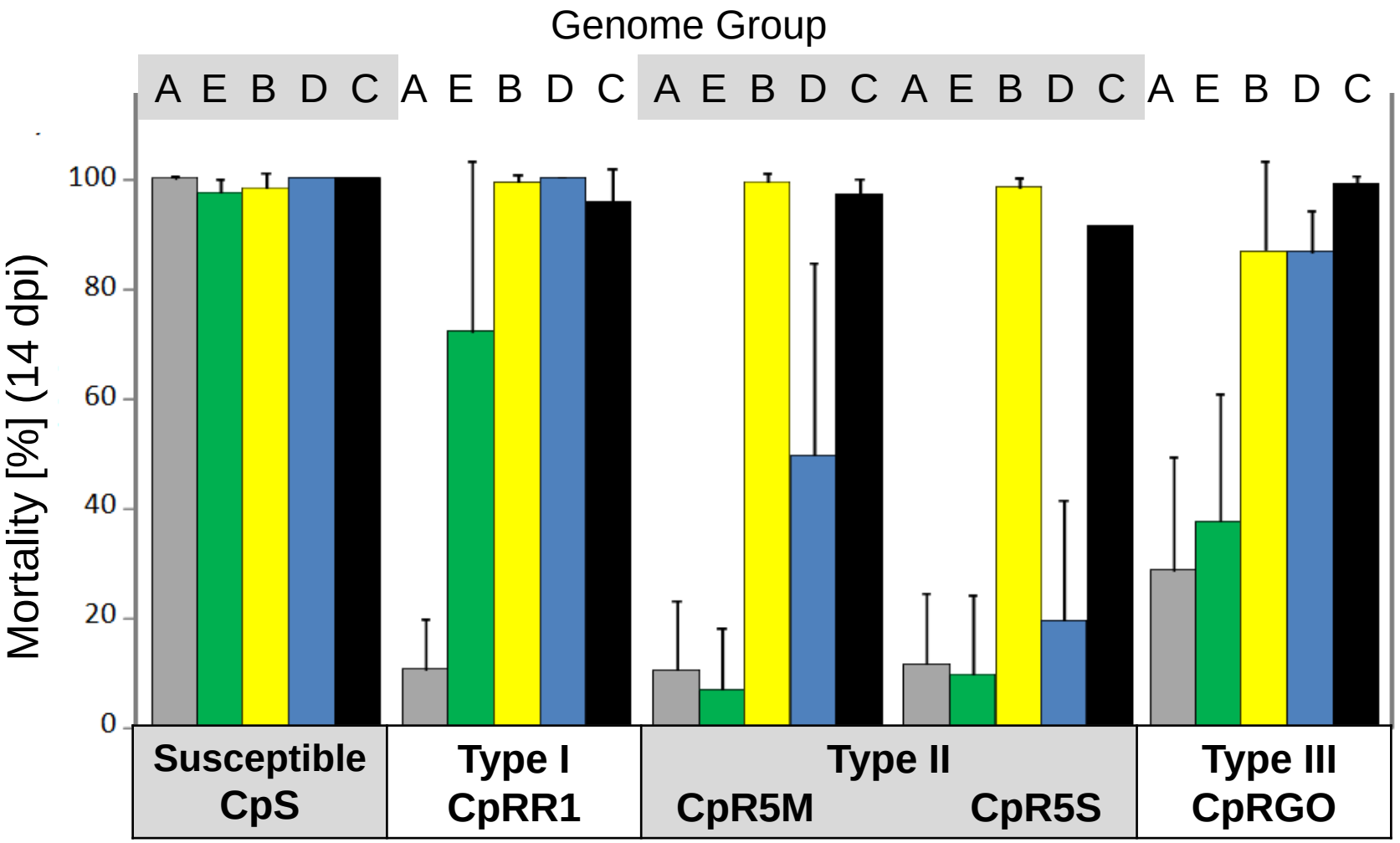
Germany (>40, type I and II)
France (>2)
Switzerland (2)
Italy (5)
Austria (1)
Netherlands (2)
Czech Republic (1)

Schmitt *et al.* JEN 2013; Zichova et al. JEN 2013; Sauer et al. PLOS 2017, AEM 2017

New commercial selections of CpGV isolates

- **MadexPlus,** (Andermatt Biocontrol, Switzerland) (registered since 2008), **Cyd-X HP** (US)
- **Carpovirusine Evo2** (Arysta-NPP, France), registered since 2012
- **MadexMax** (Andermatt Biocontrol), first registration 2010
- **MadexTop** (Andermatt Biocontrol), registration 2013
- **MadexTwin** (EU), **MadexHP** (US), **Carpovirusine PLUS** increased efficacy against OFM

Isolate dependent efficacy in different resistance types



Characterizing natural CpGV isolates

Illumina NextSeq500

- ~2.5 million paired end reads per isolate
- Each read 151 bp in length
- Up to 3,000-fold coverage of each genomic position
- Detection of SNP patterns and virus mixtures
- Genomes of >20 CpGV isolates sequenced

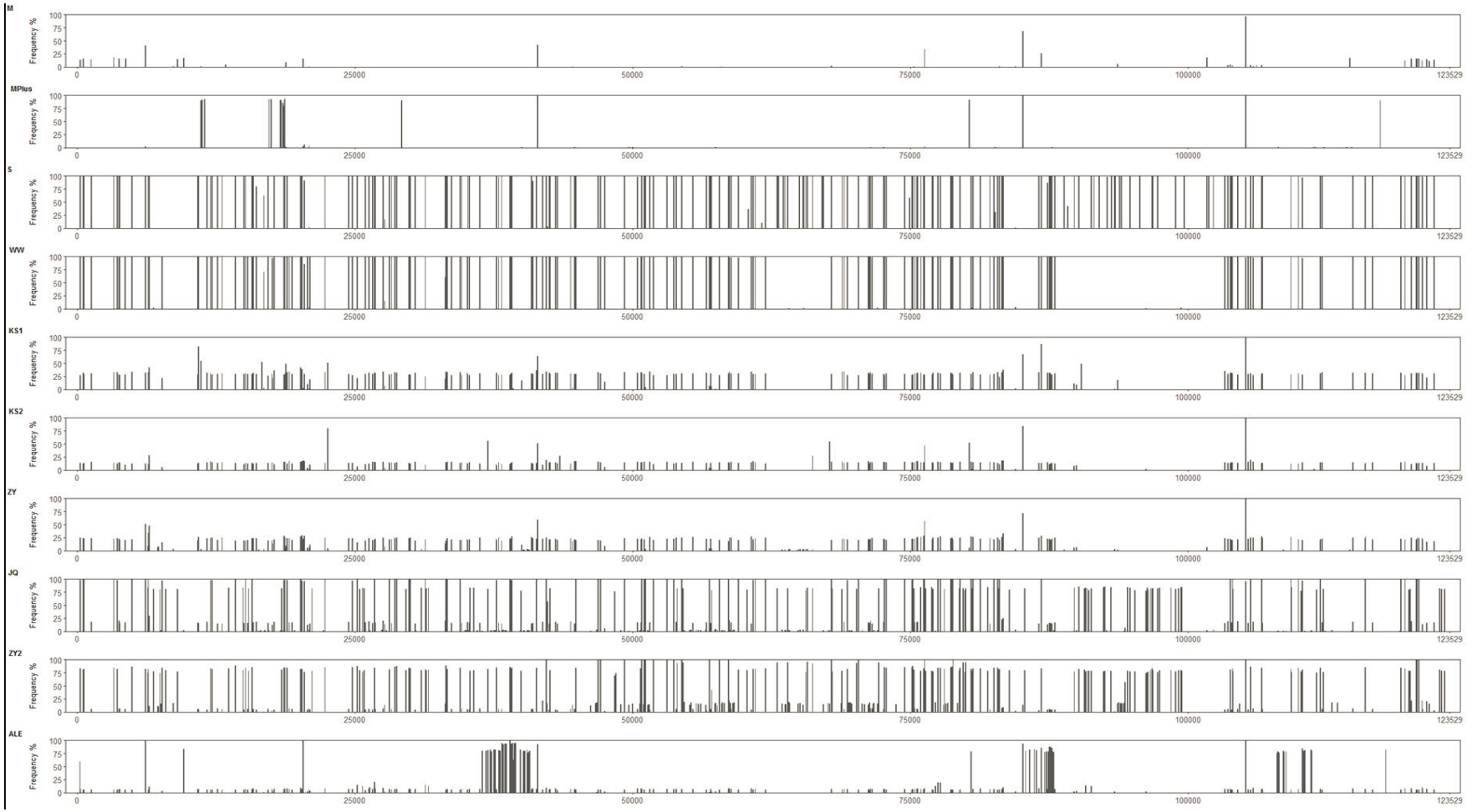
✓ **Many isolates are genotype mixtures**

✓ **Phylogenetic models do not apply for mixtures**

✓ **Bioinformatic pipeline for isolates´ genome analysis**

SNP Fingerprint
High Resolution Mapping

Fan et al. 2020 (Virology 541, 32-40)



Summary and Conclusion I

- Baculoviruses are highly specific and effective biocontrol agents
- Host specificity is the consequence of 140 Million years of co-evolution with insect hosts
- Host specificity and host range depend on successful virus-host interaction during replication
- Many factors needed for a successful infection, one factor missing may result in block of replication
- Baculoviruses are highly compatible with other control measures
- Ideally suited for IPM in organic and conventional production
- Because of host specificity, indications of a single virus are highly restricted, often to a single pest species
- Therefore, many baculoviruses do not go to registration and application

Summary and Conclusion II

CpGV resistance and strain specificity

- Five (seven) phylogenetic lines of CpGV (A-G) known for CpGV
- Three different types of resistance (type I, II, III) identified
- Molecular mechansims of resistance still not unterstood
- Commercial resistance-breaking CpGV isolates are currently available by exploiting the genetic diversity of CpGV
- In depth-knowledge into genetic diversity of CpGV by NGS sequencing allows optimization and design of products

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