prognosis of CpGV resistant populations in the field. However, disadvantages are that only few insects can be tested without any repetition of trials. Thus, both methods have pros and cons: for detailed analysis method A is superior, if fastness and high-throughput is needed, then method B is more efficient.

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The genomic variety of CpGV isolates: comparison of four genotypes

The *Cydia pomonella* Granulovirus (CpGV) is an economically important agent for the biological control of codling moth (*Cydia pomonella*, CM). Recently, the emergence of CM populations highly resistant to CpGV products (Science 317, 1916-18 (2007)) as well as the identification of new CpGV isolates overcoming CpGV resistance (J. Invertebr. Pathol. 98, 293-98 (2008)) were reported. Here we describe the genome sequencing and comparative genomic analyses of CpGV isolates vulnerable to resistance (CpGV-M) and of isolates overcoming resistance (CpGV-I12, -S) as well as an isolate with reduced virulence to susceptible CM larvae (CpGV-I07).

The isolate CpGV-M1 was one of the first fully sequenced granulovirus genomes. By restriction fragment length polymorphism (RFLP) analysis, further CpGV isolates had been previously identified and were designated due to their geographic origin. Applying phylogenetic analysis of ten CpGV isolates based on the polyhedrin/granulin (polh/gran) and late expression factor-8 (lef-8) genes, CpGV isolates could be recently grouped into genome types A to E, replacing the previous classification. To gain insight into the genomic variety and plasticity of CpGV genomes, four CpGV genome types were completely sequenced: CpGV-I12 (type D genome), -S (type E genome), -I07 (type C genome) and compared to CpGV-M (type A genome), which was re-sequenced as reference. Genome analysis revealed differences in genome size and genetic content between the four isolates. Several insertions and deletions ranging from few nucleotides to 2.5 kbp were found, concerning non-coding as well as putative coding regions. Regarding the site of these indel mutations, it is striking that the genome regions between 18-22 kbp and 50-60 kbp reveal a multiplicity of insertions, deletions and duplication events when comparing the four genomes, suggesting that these events are associated with the homologous repeat (hr) regions. Analysis of these genomic rearrangements, open reading frame (ORF) content and codon usage give insight into the evolutionary forces driving the micro-evolution of baculovirus genomes. As type D and type E genome overcome the previously described resistance of codling moth to CpGV), the comparisons of the four genomes revealed first evidence for the molecular factors involved in the virulence of CpGV to susceptible and resistant codling moth.

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Development of a PCR based method for identification, discrimination and quantification of baculoviruses specific for cutworms, *Agrotis* sp.

Cutworms of the species *Agrotis segetum* and *A. ipsilon* (Lepidoptera, Noctuidae) are serious pest insects in Africa, Europe and Asia, as they feed on many field crops and vegetables.

In the past, four baculoviruses were isolated from *A. segetum* and *A. ipsilon* larvae and characterized on molecular level: Two nucleopolyhedroviruses (NPVs) were isolated from *A. segetum* larvae in Poland (AgseNPV-A) (J. Invertebr. Pathol. 90, 64-8 (2005)) and United Kingdom (AgseNPV-B) (Arch. Virol. 75, 43-54 (1983)), one AgipNPV (J. Invertebr. Pathol. 74, 289-294 (1999)) was found in *A. ipsilon* larvae and a granulovirus (AgseGV) was also isolated from *A. segetum*. Bioassays showed that both cutworm species are susceptible to all AgseNPV-A, -B, AgipNPV and AgseGV. To develop an environmentally safe biocontrol agent the narrow host range of baculoviruses is one of their advantages. For resistance management, however, the usage of a combination of different baculoviruses is regarded to be useful. Both requirements make AgseNPV-A, -B, AgseGV and AgipNPV excellent candidates as agents for the biological control of cutworms. In order to discriminate the different *Agrotis*-specific baculoviruses a reliable method for identification and quantification is essential.

In this work, we focused on the optimization of AgseNPV and AgseGV purification protocols and show that the yield of NPVs and GVs in mixed infections depends on the established purification method. Furthermore, multiplex polymerase chain reaction (PCR) and quantitative real time PCR (qRT-PCR) based methods were established allowing the specific amplification of discriminating fragments of their polyhedrin (polh) and granulin (gran) genes (fragment lengths: AgseNPV-A 199 bp, AgseNPV-B 263 bp, AgseGV 347 bp and AgipNPV 527 bp). Thus, a rapid and robust method to detect the amounts of AgseNPV-A, -B, AgseGV and AgipNPV in mixed infections becomes possible. It also provides an important tool in the quality control of production of baculoviruses specific for *Agrotis* species.