Stability over passages on genotype frequencies and pathogenicity of CpGV mixed genotype populations

Aurélie Hinsberger, Benoit Graillot, Christine Blachère-Lopez, Miguel Lopez-Ferber

Stability over passages on genotype frequencies and pathogenicity of CpGV mixed genotype populations

Aurelie Hinsberger[1], Benoit Graillot[1], Christine Blachère-Lopez[1,2], and Miguel Lopez-Ferber[1]
[1]Hydrosciences Montpellier, Univ Montpellier, IMT Mines Ales, IRD, CNRS, 6 Avenue de Clavières, Alès, France; [2]INRAE, SPE., Rte des Chappes, 06560 Sophia Antipolis, France
Correspondence: miguel.lopez-ferber@mines-ales.fr:

Abstract: Cydia pomonella granulovirs isolate R5 is able to replicate in codling moth larvae susceptible or resistant to the Mexican isolate of CpGV. An experimental virus lineage was constructed by mixing 99 % CpGV-R5 and 1 % CpGV-M. This lineage was replicated for 20 passages on susceptible insects at high multiplicity. The relative proportion of CpGV-M in this lineage increases over passages, becoming predominant after the 10th passage. However, this virus lineage retains its ability to replicate on R_{GV} insects, that are resistant to CpGV-M. It has been previously demonstrated that in genotypic mixtures CpGV-R5 acts as helper of CpGV-M for replication on R_{GV} larvae. The increasing proportion of CpGV-M over passages suggest that a previously undetected selective advantage exists for CpGV-M when replicating in epizootic conditions but reveals also that CpGV-R5 does not disappear quickly from the virus population.

Key words: Cydia pomonella granulovirus, synergy, codling moth control

Introduction

Codling moth, *Cydia pomonella* (L.), is a major pest of apples and pears distributed worldwide in temperate climates. Massive use of chemicals against this pest progressively led to the emergence of resistance to most of them. In parallel, other methods were developed to control this pest.Among them, the Cydia pomonella granulovirus (Baculoviridae, Betabaculovirus). In Europe, CpGV use developped since the first registration in 1988, reaching more than 100 000 ha. All commercial formulations before 2010 were derived from the first discovered Cydia pomonella granulovirus (CpGV) isolate, CpGV-M (Lacey et al., 2008).

Resistance to CpGV-M products has been reported in Germany and France from 2005. All resistant populations examined appear to harbor similarly inherited resistances (Schmitt et al., 2013). This resistance -called type I resistance- is specific against CpGV-M. It is controlled by a single locus located on the Z chromosome of the moth (Asser-Kaiser et al., 2010). The highly resistant (type I) R_{GV} insect colony was obtained from a natural population of south France by selection to near allele fixation and adaptation to laboratory conditions. It presents a caracteristic resistant ratio of 10,000 when challenged with CpGV-M, compared to susceptible colonies (Berling et al., 2009).

Various CpGV isolates are able to control type I resistant insects. Among them, CpGV-R5 (Graillot et al., 2014). Gebhardt et al. (2014) analyzed representative virus isolates differing in their ability to overcome resistance. They demonstrated the link between an allele of the *p38* virus gene and the ability to replicate in resistant insects, a 24bp insertion in the *pe38-M* allele compared to the resistance breaking allele, *pe38-R*.

Fitness analysis were carried out both at the host and at the virus levels. No detectable fitness cost was found in laboratory conditions for insects carrying type I resistance (Undorf-Spahn et al., 2012). From the virus perspective, no statistically significant differences were found on the OB production levels between CpGV-M and CpGV-R5 (Graillot et al., 2014). In addition, an analysis of the stability of CpGV-R5, during successive passages on susceptible larvae could complement such studies.

To that aim, we replicated CpGV-R5 for 20 cycles on susceptible insects at high multiplicity of inoculation, mimiking epizootic conditions. Then, we analyzed both their genetic composition at the level of the *p38* virus gene, and their ability to replicate and control the resistant laboratory colony R_{GV}.

Materials and methods

Insects and viruses

Two laboratory colonies of *Cydia pomonella* were used in this study, CpNPP and R_{GV}. The colony CpNPP is susceptible to both CpGV-M and CpGV-R5, while the R_{GV} colony harbours the major genetic determinant for resistance present in resistant European populations. Both insect colonies are reared on artificial diet as previously described (Berling et al., 2009)

Virus isolates used in this work, CpGV-M and CpGV-R5 have been described previously. CpGV-R5 is able to overcome the resistance of the R_{GV} colony and results in a productive lethal infection in resistant insects. No polymorphisms were detected by restriction enzyme length polymorphism analysis in this virus isolate (Graillot et al., 2014). An experimental genotype mixture was made by mixing 1 % of CpGV-M and 99 % of CpGV-R5 OBs (1M:99R), and used to start a virus lineage by amplifiying on susceptible CpNPP larvae.

Virus amplification and successive passages on the susceptible host colony

Virus amplifications were performed as previously described (Graillot et al., 2016). Larvae inoculated with 1M:99R showing clear signs of infection at 4 dpi were collected and placed in groups of three into microfuge tubes and incubated one more day at 25 °C, then frozen (-20 °C). For OB extraction, larvae were thawed, pooled and homogenized in distilled water, then filtrated through nylon cloth to eliminate debris. The suspension was then centrifuged 5 min at 8,000 × g, and the resulting pellet was re-suspended in distilled water. This suspension constituted the first amplification (R5-S1) of the virus. The purified OB concentrations were evaluated after each passage, adjusted to 800 OB/µl and used as inoculum for the following passage. Successive amplifications have been performed up to the 20th generation, called R5-S20.

Bioassays

Quantitative bioassays were performed using established protocols using neonate larvae of both insect colonies CpNPP and R_{GV} (Berling et al., 2009). Virus induced mortality was recorded after seven days. Mortality data were subjected to probit analysis using the POLO+ software (LeOra, 1987). Each bioassay was repeated three times. The results for each treatment were pooled after control of their homogeneity.

DNA extraction, PCR amplification and HRM analysis

Lysis of OBs was performed in order to liberate viral DNA from 10 of the OB suspensions obtained previously (R5, R5-S1, R5-S3, R5-S5, R5-S8, R5-S10, R5-S13, R5-S15, R5-S18, R5-S20) and the 99M:R1 experimental mixture. qPCR was carried out using CFX96 Touch™

Real-Time PCR Detection System (Bio-Rad Laboratories), followed by HRM as previously described (Hinsberger *et al*., 2019). Biorad CFX maestro™ and Precision Melt Analysis™ were used for data analysis.

Results and discussion

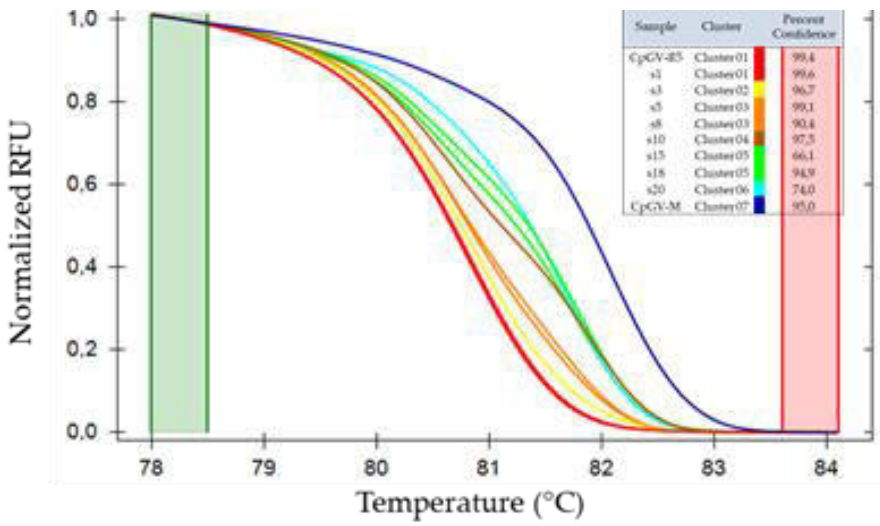
CpGV genotypes able to control type I resistant insects were not present in CpGV-M isolate. This could be the reflection of a cost for the virus to maintain the ability to bypass resistance or just be the consequence of a founder effect as CpGV-M comes from a limited number of larvae. Once resistance became a problem, resistance-breaking genotypes were identified both by sampling for new isolates in various locations, or by selection from other stored isolates. Among them, the CpGV-R5 isolate.

Assays of mixed infections in the same host suggested a synergistic effect at least in some conditions (Graillot et al., 2016). The frequency of each genotype over generations depends both on the host genotype, and on the epizootic conditions (Graillot et al., 2019).

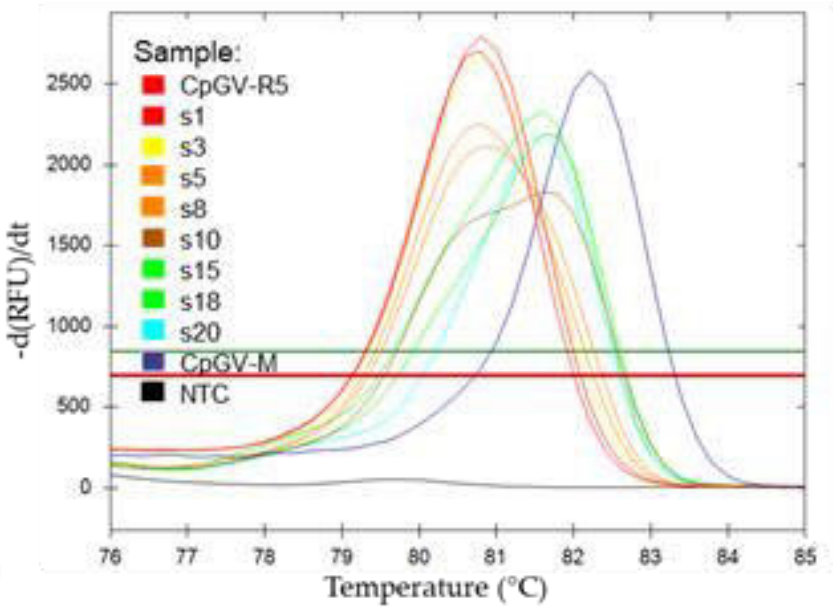
To identify a putative selection of virus genotypes, twenty successive passages have been carried out on susceptible insects CpNPP (R5-S1 to R5-S20), starting with a virus population obtained by mixing 1 % CpGV-M and 99 % CpGV-R5. The evolution of this virus lineage was followed by analysing the genetic composition at the level of the *pe38* gene by PCR followed by HRM, using a couple of primers allowing differentiating between *pe38-M* and *pe38-R* alleles (Hinsberger et al., 2019).

The HRM analysis identifies 7 different clusters (Figure 1 a). Only sample R5-S1 clusters with the parental population. From passage 3, the virus lineage tends to CpGV-M, the melting profile adopting a two peak profile (R5-S10) or a single intermediate profile R5-S15 to R5-S20 (Figure 1 b). Figure 1 c presents the data from the same experiment plotted relative to the RFU of the CpGV-R5. The height of the peak is proportional to the CpGV-M genotype frequency, confirming the increasing prevalence of CpGV-M genotypes in the lineage.

a



b



c

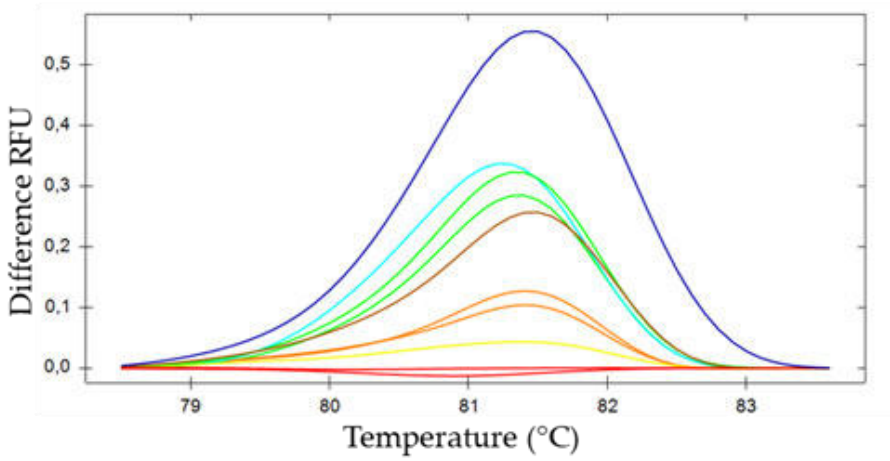


Figure 1. Melting curves analysis of amplicons from an experimental CpGV population composed of two genotypes, CpGV-M and CpGV-R5, amplified on susceptible insect for the number of passages indicated. (a) Normalized RFU (b) First derivative of fluorescence vs temperature profile (c) Plot of deviations on RFU relative to the (CpGV-R5) (in red), used as reference.

Amplicons of different passages were checked by electrophoresis and sequencing to verify if recombination occurs. The sizes and sequences correspond to those expected for CpGV-M and CpGV-R5.

The pathogenicity of this lineage has been determined at passages 0 (corresponding to 1 % M:99 % R5), R5-S5, R5-S10, R5-S15 and R5-S20 on R_{GV} and CpNPP. A limited variation in the ability to control the resistant insect colony is observed, far lower than expected if the resistance trait was being lost (Figure 2).

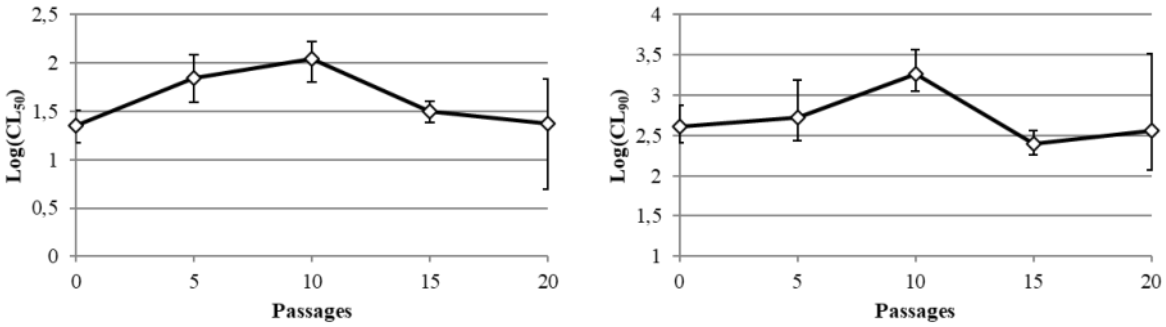


Figure 2. Changes of efficacy of the virus lineage on neonate resistant larvae R_{GV} after passages on susceptible larvae CpNPP. The efficacy is measured as LC_{50} or LC_{90}.

These results suggest that in conditions without selective pressure for maintaining the ability to replicate in resistant insects, the CpGV-M genotype presents a higher fitness. However, this advantage is low, as CpGV-R5 genotype is still present after 20 generations, and the ability to control resistant populations did not significantly drop during the whole experiment. This maintain may be due to the observed synergy when both viruses infect the same larvae, that is, in conditions of high prevalence. However, in conditions of low prevalence, the probability of such mixed infections drops, resulting in a progressive increase of the small differences in the final proportion of each genotype at each generation. The fact that in the Mexican isolate no genotypes able to control type I resistant insects were found is likely the result of a founder effect or of replication at low multiplicity conditions.

Acknowledgements

This study received funding from the European Commission Seventh Framework Programme (FP7/2007-2013), under grant agreement FP7-265865. Benoit Graillot received an Agence Nationale de la Recherche Technologique doctoral scholarship. Aurelie Hinsberger received a Region Occitanie doctoral scholarship. The authors want to thank A. Bezier for critical comments on the manuscript.

References

Asser-Kaiser, S., Heckel, D. G. and Jehle, J. A. 2010. Sex linkage of CpGV resistance in a heterogeneous field strain of the codling moth *Cydia pomonella* (L.). J. Invertebr. Pathol. 103: 59-64.

Berling, M., Blachere-Lopez, C., Soubabere, O., Lery, X., Bonhomme, A., Sauphanor, B. and López-Ferber, M. 2009. Cydia pomonella granulovirus genotypes overcome virus resistance in the codling moth and improve virus efficiency by selection against resistant hosts. Appl. Environ. Microb. 75: 925-930.

Gebhardt, M., Eberle, K. E., Radtke, P. and Jehle, J. A. 2014. Baculovirus resistance in codling moth is virus isolate-dependent and the consequence of a mutation in viral gene pe38. Proc. Natl. Acad. Sci. USA 111: 15711-15716.

Graillot, B., Berling, M., Blachère-López, C., Siegwart, M., Besse, S. and López-Ferber, M. 2014. Progressive adaptation of a CpGV isolate to codling moth populations resistant to CpGV-M. Viruses 6: 5135-5144.

Graillot, B., Bayle, S., Blachère-López, C., Besse, S., Siegwart, M. and López-Ferber, M. 2016. Biological Characteristics of Experimental Genotype Mixtures of Cydia pomonella Granulovirus (CpGV): Ability to Control Susceptible and Resistant Pest Populations. Viruses 8: 147, doi:10.3390/v8050147.

Graillot, B., Blachère-López, C., Besse, S., Siegwart, M. and López-Ferber, M. 2019. Importance of the Host Phenotype on the Preservation of the Genetic Diversity in Codling Moth Granulovirus. Viruses 11: 621; doi:10.3390/v11070621.

Hinsberger, A., Theulier Saint Germain, S., Guerrero, P., Blachère-López, C., López-Ferber, M. and Bayle, S. 2019. A Combination of Real-Time PCR and High-Resolution Melting Analysis to Detect and Identify CpGV Genotypes Involved in Type I Resistance. Viruses 11: 723, doi:10.3390/v11080723.

Lacey, L. A., Thompson, D., Vincent, C. and Arthurs, S. P. 2008. Codling moth granulovirus: A comprehensive review. Biocontrol. Sci. Techn. 18: 639-663.

LeOra 1987. Polo-pc software. A user's manual for Probit or Logit analysis. LeOra Software. Berkeley, CA.

Schmitt, A., Bisutti, I. L., Ladurner, E., Benuzzi, M., Sauphanor, B., Kienzle, J., Zingg, D., Undorf-Spahn, K., Fritsch, E., Huber, J., and other authors 2013. The occurrence and distribution of resistance of codling moth to Cydia pomonella granulovirus in Europe. J. Appl. Entomol. 137: 641-649.

Undorf-Spahn, K., Fritsch, E., Huber, J., Kienzle, J., Zebitz, C. P. and Jehle, J. A. 2012. High stability and no fitness costs of the resistance of codling moth to Cydia pomonella granulovirus (CpGV-M). J. Invertebr. Pathol. 111: 136-142.