Does gender affect to SeMNPV vertical transmission in *Spodoptera exigua*?
Vertical transmission of SeMNPV highly consistent throughout female linage parental

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Abstract: Vertical transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) is a common feature in field *S. exigua* populations. To assess whether the gender affect to transgenerational virus transmission or distribution four mating groups were performed: i) healthy males (H♂) × healthy females (H♀); ii) infected males (I♂) × healthy females (H♀); iii) healthy males (H♂) × infected females (I♀) and iv) infected males (I♂) × infected females (I♀). These adults and their offspring were analyzed by qPCR to detect SeMNPV infection. Both males and females were able to transmit the infection to the next generation, although females infected high percentage of the offspring and their transmission was more stable and consistent. Venereal transmission is not likely to occur, and the main route of transmission seems transovarial more than transovum. The prevalence of the infection in the offspring did not vary according to gender, therefore both males and females can be infected by their parents in similar proportion. In conclusion, SeMNPV vertical transmission occurs by paternal and maternal via, while females transmission is more consistent. This route of transmission can improve the development of the bioinsecticides based in NPVs.

Key words: SeMNPV, covert infection, gender, transgenerational transmission

Introduction

Baculoviruses are the most extensively studied arthropod-specific virus due to their extremely high virulence to worldwide insect pest and for their good genomic characteristics for mass-production of recombinant proteins. Because of their high specificity, persistence and safeness to non-target organisms (including humans), they have been developed as insect pest control agents against important pest for crops and forests protection (Moscardi, 1999). Dynamics of baculovirus population involve two transmission pathways to colonize and survive in the host. Occlusion bodies (OB) are responsible of the between larvae (horizontal) transmission, that is thought to be major pathway of baculovirus transmission. Little is known about vertical transmission of viruses in insects but it has been proposed as a survival strategy to overcome periods of host population scarcity and to facilitate the virus dispersal to geographically spaced niches. Transgenerational transmission involves covertly infected adults that pass virus to their progeny via the transovum or transovarial pathway (Cory & Myers, 2003). A previous study showed that *Spodoptera exigua* females with no evidence of a nucleopolyhedrovirus (NPV) covert infection produced virus-infected offspring. This led us to suspect that both sexes may contribute to vertical transmission of the pathogen. However, the differential prevalence of covert infection between males and females (Cabodevilla *et al*., 2011), suggests a possible gender effect on the transmission mechanisms. In this study we

analyzed the effect of gender on the transmission efficiency of covert infections by *S. exigua* nucleopolyhedrovirus (SeMNPV) to their progeny.

Material and methods

Insect and virus

The experiment was performed with a virus-free laboratory culture of *S. exigua*. A single genotype of SeMNPV, named VT-SeAl1, was used in the experiment. This genotype was previously isolated from a sublethally infected culture of insects collected in the greenhouses of Almeria (Spain) and thus transmitted from parents to offspring.

DNA extraction and detection of covert infections

Total DNA was extracted from the abdomens of the adults after being sexed by the observation of the external genitalia. Quantitative PCR based on SYBR fluorescence was carried out to detect SeMNPV infection. Specific primers were designed to amplify a 149-bp region of the DNA polymerase gene (DNApol149-Fw: 5'-CCGCTCGCCAACTACATTAC-3'; DNApol149-Rv: 5'-GAATCCGTGTCGCCGTATATC-3') based on the complete genome sequence of the SeMNPV strain VT-SeAl1 (unpublished data). For the standard curve VT-SeAl1 DNA was extracted from OBs, purified thorough CsCl gradients, quantified using a spectrophotometer and then serially diluted in sterile MilliQ water up to the following concentrations: 10, 1, 0.5, 0.1, 0.05, 0.01, 0.005, and 0.001 pg/µl). Quantified viral DNA was normalized based on the total DNA concentration for each sample and measured using a NanoDrop 2000.

Bioassays

To determine gender influence on vertical transmission of the SeMNPV, groups of adults either sublethally infected (infected males: I♂ and infected females: I♀) or virus-free adults (healthy males: H♂ and healthy females: H♀) were required. For that purpose, two subpopulations genetically identical were generated by inducing sublethal infections artificially on a virus-free insect culture (qPCR detection limits 10^{-3} pg of viral DNA). Two hundred *S. exigua* fourth instar larvae were treated with 9 x 10^3 OB/ml suspension, in parallel a group of 100 larvae were treated in the same conditions but without OB suspended. Surviving insects were reared, sexed and then classified in separate groups according to their sex and viral treatment. Once the adults emerged, we set up the following mating schedule: i) healthy males (H♂) × healthy females (H♀); ii) infected males (I♂) × healthy females (H♀); iii) healthy males (H♂) × infected females (I♀) and iv) infected males (I♂) × infected females (I♀). Five adult pairs were confined in paper bags for oviposition. Eggs batches from each treatment group were harvested and the adults frozen at -80°C for subsequent analysis (F_0 generation). Egg masses laid from each paper bag were divided into two parts, and either soaked in a 0.25 ppm hypochlorite solution (surface decontamination) or in distilled water (no decontamination) for five minutes. Twenty-five neonates were individually reared on semi-artificial diet through to adult stage (F_1) and then stored at -80ºC for subsequent analysis. The whole procedure was performed four times.

Results and discussion

Establishing sublethal infection and qPCR parameters

The 57.6 ± 4.4 % of the larvae initially exposed to VT-SeAl1 succumbed to virus infection, while no mortality was registered in mock-infected control larvae.

The efficiency and the sensitivity for the qPCR reaction were evaluated. The line parameters and the regression coefficient confirmed the goodness of fit (R^2 0.995) and high efficiency of the reactions (slope= -3.215). The detection limit for this trial was 1×10^{-3} pg, as the genome of VT-SeAl1 was estimated to be 135696 bp (unpublished data) equates theoretically to 6.83 viral genome copies.

The frequencies of qPCR positive in the survivors to a virus challenged were far higher than those measured in control insects ($\chi^2 = 60.49$, $df = 1$, $P < 0.001$) (Figure 1.A). Viral load in F_0 parental adults averaged $1.514 \pm 0.287 \times 10^{-3}$ pg viral DNA/µg total DNA per insect (N=72, positives for qPCR) that correspond to 10.34 ± 1.96 genome copies per adult.

A very few adults that did not exposed to virus but mated with infected adult were positive to qPCR analysis (Figure 1.A). However, qPCR positives frequency in the control group H♂ × H♀ (5/50) was similar to that found in apparently healthy groups mated with infected insects (I♂ × H♀ = 4/25 and H♂× I ♀ = 3/25; $\chi^2 = 0.379$, $d.f = 1$, $P = 0.538$). Therefore it seems unlikely that venereal infections occurred but punctually cross-contamination during the insects rearing.

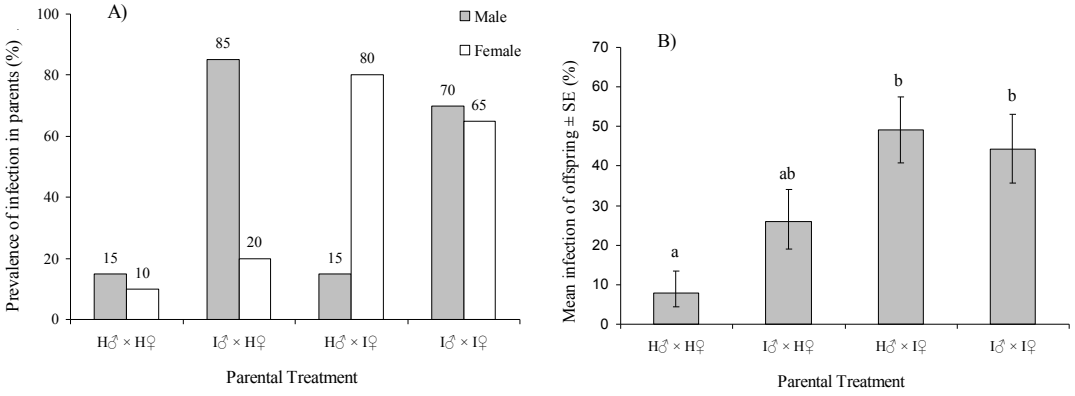

Figure 1. A: Prevalence of infection in parental adults for mating groups. Twenty five male and females were included per mating group. B: Percentage mean of *S. exigua* adults positive by qPCR in the offspring for parental treatment (N=120). Columns labeled with different letters denoted significant differences by the *t*-test ($P < 0.05$). H♂: Healthy male, H♀: Healthy female, I♂: Infected male, I♀: Infected female.

Virus transgenerational transmission

The prevalence of virus in F_1 adults (% of positive qPCR) was compared between adults from either decontaminated or non-decontaminated eggs. Eggs surface decontamination treatment did not significantly affect the percentage of F_1 adults sublethally infected ($\chi^2 = 0.649$, $df = 1$, $P = 0.420$). This results are in agreement with more recent studies made with *Spodotera exempta* nucleopolyhedrovirus reporting that surface decontamination of eggs did not affect the detection of the virus in the offspring of infected insects (Vilaplana *et al*., 2008), suggesting that transovarial transmission represent the most likely pathway for the virus to infect descendants.

Both male and female adults sublethally infected were capable to transmit the infection to descendants (Figure 1.B). Interestingly only female linage consistently transmitted virus infection to their offspring, being the prevalence of infection in the offspring of mating groups

involving infected female significantly higher than healthy controls (*t*-test, $P < 0.05$). However, the response observed in the offspring from infected male lineage, highly varied and did not significantly differed to the offspring from either the control healthy parental lineage or the both-part infected lineage mating groups. Some studies with *Droshopila* sigma virus, indicate that transmission rates are greater in females than males of *D. obscura* and *D. affinis* (Longdon *et al.* 2011), despite of transmission occur through both eggs and sperm. Studies conducted on baculovirus transmission demonstrated that both sexes were involved in vertical transmission for *Plodia interpunctella* granulovirus, they found viral particles in either testis or ovaries cells, confirming the presence of the virus in gonads of sublethally infected individuals by viral transcript detection (Burden *et al*., 2002).

Mean values of viral load in F_1 adults were similar between mating groups ($F = 1.31$, $df = 3, 12$, $P = 0.316$) and ranged from $1.07 \pm 0.12 \times 10^{-3}$ to $1.76 \pm 0.29 \times 10^{-3}$ pg viral DNA/ µg total DNA, therefore the quantity of viral DNA per sublethally infected insect was independent of the parental lineage passing on the virus (male, female or both). Contrary, Longdon *et al.* (2011) detected less titters of *D. affinis* sigma virus and *D. obscura* sigma virus in the embryos when the virus was paternally transmitted.

The distribution of the virus in the F_1 generation is not biased by gender, being equal likely to be infected male than female ($F = 0.997$, $df = 1, 28$, $P = 0.327$).

Finally a linear correlation was proved between viral load per F_1 infected insect and the proportion of F_1 infected insects, so the more likely of getting infections the higher titters found in those adults (spearman rank correlation: 0.687, $P < 0.05$).

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