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Fitness-related traits of entomopoxviruses isolated from *Adoxophyes honmai* (Lepidoptera: Tortricidae) at three localities in Japan

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ABSTRACT

Three entomopoxviruses (EPVs) isolated from diseased *Adoxophyes honmai* larvae at different localities (Tsukuba, Itsukaichi, and Miyazaki) in Japan were compared for biochemical identity and key parameters of virus fitness, fatal infection, speed of kill, and virus yield. When the structural peptides of occlusion bodies (OBs) and occlusion-derived viral particles were compared using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, no difference in banding patterns was observed. However, DNA restriction endonuclease analysis showed that the three isolates were genotypically different, but many commonly sized DNA fragments were observed. Five tortricid species, *A. honmai*, *Adoxophyes orana*, *Adoxophyes dubia*, *Homona magnanima*, and *Archips insulanus* were susceptible to all isolates. No significant differences in the key viral fitness parameters were detected among the isolates in *A. orana*. However, the Miyazaki isolate had a different effect on *H. magnanima*; it allowed infected insects to survive longer and develop to a larger size, but had a lower yield of OBs per larva at any given time to death. OB yields per unit cadaver weight for the Miyazaki isolate, which indicate the conversion rate of the insect to virus, were lower over time compared to the other two isolates. The implications for selecting a candidate isolate to control tortricid pests are discussed.

1. Introduction

Entomopoxviruses (EPVs) are large oval-shaped viruses with a linear double-stranded DNA genome and a molecular weight of 200–240 kbp. EPVs belong to one of two *Poxviridae* subfamilies: *Entomopoxvirinae*, which are insect poxviruses, or *Chordopoxvirinae*, which are vertebrate poxviruses (King et al., 1998). *Entomopoxvirinae* is divided into three genera according to viral morphology, host range, and genome size: *Alphaentomopoxvirus, Betaentomopoxvirus*, and *Ganmaentomopoxvirus*. *Alphaentomopoxvirus* comprise viruses infecting coleopterans, *Betaentomopoxvirus* infects lepidopterans and orthopterans, while those from *Ganmaentomopoxvirus* infect dipterans (Buller et al., 2005). Although recent sequencing studies have shown that all members of the two *Poxviridae* subfamilies have several conserved genes, EPVs are believed to be specific to insects. EPV virions are embedded in occlusion bodies (OBs) or spheroids composed mainly of a highly expressed protein called spheroidin. After ingestion by the insect host, OBs are dissolved by the alkaline-reducing conditions in the midgut and release virions, resulting in viral replication in the midgut epithelial cells, followed by transmission to the internal tissues. Deleting the spheroidin gene has no effect on viral replication *in vitro* (Palmer et al., 1995); however, spheroidin should have an important function in the viral life cycle in nature by protecting virions from environmental asperity, similar to the polyhedrins of baculoviruses or cypoviruses. EPV pathology depends on the insect host, but the infection course is generally slow (Arif, 1995). For example, larvae of the lepidopteran *Choristoneura fumiferana* infected with *C. fumiferana* EPV show few symptoms until late in the infection and die in 1–3 weeks (Palli et al., 2000). The EPV-infected orthopteran *Locusta migratoria* shows a decreased developmental rate and eventually dies 20–60 days after infection (Jaeger and Langridge, 1984). In the dipterans, *Chironomus attenuatus* and *Goeldichironomus haloprasimus*, EPV-infected larvae appear to survive up to 8 weeks after infection (Huger et al., 1970). EPV-infected coleopterans may survive for up to 40 weeks (Goodwin and Roberts, 1975).

The potential use of EPVs as microbial control agents has attracted attention (Mason and Erlandson, 1994; Wegensteiner et al., 1996; Woods et al., 1992). Microbial control agents have been used for integrated pest-management programs with differing strategies (Fuxa, 1987). For example, a pathogen that can kill its insect host shortly after infection could be used in place of a chemical insecticide. In contrast, an appropriate use of a pathogen

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that needs considerable time to kill its insect host, such as EPVs, may reduce the population growth rate of the insect pest via epizootics. Even small decreases in the population growth rate can affect insect outbreak frequency and, thereby, diminish the need for chemical insecticides (Throne, 1989). This strategy relies on natural epizootics, so it requires persistence and efficient transmission in the target insect pest population and ecosystem. Before applications in nature, it is useful to know the phenotypic characteristics related to viral fitness under laboratory conditions, such as infectivity, kill speed, and viral yield in the target insect pest. Such characteristics pertain to the capability to cause epizootics, which is important in assessing the viral agents for use in the control strategy under consideration. Additionally, comparing viral isolate fitness-related traits aids in the selection of a candidate isolate for insect pest control.

Here, we describe an experiment in which we examined the fitness-related traits of three EPV isolates (hereafter, AdhoEPV) from the smaller tea tortrix, *A. honmai*, from different localities in Japan to select good potential isolate(s) as a control agent against tortricid insect pests, particularly orchard pests, such as the summer fruit tortrix, *Adoxophyes orana*, known as a pest of apple and the oriental tea tortrix, *Homona magnanima*, known as a pest of pea as well as tea. Some tortricid lepidopterans are the principal apple and pear orchard pests in Japan, the United States, and Europe. Although the control of these pests has depended on conventional chemical insecticides, developing insecticide resistance and concerns about product safety and environmental health require the development of new agents. In the United States and Europe, *Cydia pomonella* granulovirus (CpGV) has been developed as an alternative agent, but it is limited by its narrow host range and lack of persistence in orchards (Cross et al., 1999). These are often cited as shortcomings of microbial control agents. An AdhoEPV isolate from Miyazaki, Japan infected several orchard and tea pest species of the family Tortricidae, but infection was examined using only external EPV disease symptoms (Ishikawa et al., 1983). Furthermore, AdhoEPV disease occurs endemically, and the prevalence has reached high levels in populations of *A. honmai* and *H. magnanima* in tea fields (Nakai et al., 1997; Kunimi, unpublished data). Although the degree of persistence depends on the ecosystem, this may suggest that AdhoEPV can persist in tortricid pest populations once applied.

We also subjected the three AdhoEPV isolates to biochemical comparisons to examine their genetic relatedness and sought to determine whether genotypic variation in the related EPVs translates into phenotypic differences that can affect host–EPV dynamics. Genotypic variation and differences in pathogenicity in insect–virus populations have been well demonstrated in baculoviruses at several ecological scales, but very little is known about the evolutionary processes leading to the occurrence and maintenance of genotypic variation and the dynamics of genotypes (Cory et al., 1997). Genotypic variation in EPV populations, however, has received little attention. We hope our results will stimulate studies of genotypic and phenotypic variation in EPV populations.

2. Materials and methods

2.1. Insects

We used six species from the family Tortricidae. *A. honmai*, *A. orana*, and *Pandemis heparana* strains were supplied by Agro-Kanesho Co., Ltd. (Saitama, Japan), Nagano Fruit-Tree Experiment Station (Nagano, Japan), and Nippon Kayaku Co., Ltd. (Tokyo, Japan), respectively. The *H. magnanima*, *Adoxophyes dubia,* and *Archips insulanus* strains were originally collected from tea fields in Tokyo, Okinawa Prefecture, and Okinawa Prefecture, Japan, respectively. All colonies were maintained continuously at 25 °C with a 16-h photoperiod (16L:8D). Newly laid eggs were sterilized in 3% formaldehyde for 5 min and washed twice with sterile distilled water (SDW). Hatched larvae were fed an artificial diet (Silkmate 2S; Nosan Cooperation, Kanagawa, Japan).

2.2. Viruses

We compared AdhoEPVs from Miyazaki, Itsukaichi, and Tsukuba (Fig. 1). The Miyazaki viral stock (AdhoEPV-Mi) was kindly supplied by Dr. I. Ishikawa (Saitama Tea Experiment Station, Saitama, Japan). This stock originated from multiple diseased *A. honmai* larvae collected from a tea field in 1981. Thereafter, the stock was passed through *A. honmai* and *H. magnanima* larvae several times before it was supplied as a semi-purified suspension (I. Ishikawa, personal communication). AdhoEPV-Mi has been partially characterized according to its morphology, histopathology, and biochemical aspects, and is classified as a *Betaentomopoxvirus* (Ishikawa et al., 1983; Shimamura and Watanabe, 1986; 1988). The viruses from Itsukaichi (AdhoEPV-It) and Tsukuba (AdhoEPV-Ts) were each prepared from a single diseased larva that had been stored frozen since they were collected from tea fields in 1985 and 2000, respectively.

Each of the viruses was propagated in *A. honmai* and *H. magnanima* by dipping the eggs in a semi-purified suspension (AdhoEPV-Mi) or the macerate of SDW + the larva that died of the disease (AdhoEPV-It and AdhoEPV-Ts). Thus, six inocula (three isolates × two propagation hosts) were prepared to determine the effects of propagation hosts. Although *A. honmai* was the original host from which the three AdhoEPVs were isolated, *H. magnanima* was selected as a propagation host because high OB yields were expected; this species has the largest body size among the six tortricid species used and is easy to rear. Hatched larvae were reared on an artificial diet (Silkmate 2S), and diseased larvae were collected after death of the final instar. Viral OBs were purified by homogenization and density gradient centrifugation, suspended in 0.05 M sodium phosphate buffer (pH 7.7), and then counted using a Thoma hemocytometer (Kayagaki Irika Kogyo, Tokyo, Japan). Five counts per hemocytometer sample and three subsamples per suspension were measured to reduce counting and dilution errors. These suspensions were stored at 4 °C until use. An EPV (MyseEPV) of the rice armyworm, *Mythimna separata* (Lepidoptera: Noctuidae), which was a gift from Dr. T. Hukuhara (Nihon University), was used as a reference in the Southern blot hybridization analyses because MyseEPV also belongs to *Betaentomopoxvirus* (Xu and Hukuhara, 1992).

2.3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of viral structural proteins

Each of the purified OB suspensions was incubated with one-half volume of 2× dissolution buffer (1.6 M Na_2CO_3, 0.3 M NaCl, 0.02 M EDTA, 0.04 M sodium thioglycolate [pH 10.5]) at 37 °C for 30 min to dissolve the OB matrix. Undissolved OBs and heavy debris were pelleted by centrifugation at 1500*g* for 5 min at 4 °C. The supernatants were then centrifuged at 20,400*g* for 30 min at 4 °C to pellet the occlusion-derived virus (ODV). The partially purified ODV pellets were suspended in a small volume of TE (10 mM Tris, 1 mM EDTA [pH 8.0]) and layered onto a 40–60% (w/w) sucrose gradient. After centrifugation at 50,000*g* for 1 h, the band containing ODV was collected and washed twice with TE at 76,690*g* for 30 min and then stored at −35 °C until use. ODV and the OB matrix proteins were analyzed using SDS–PAGE according to the method of Laemmli (1970). The ODV and OB were boiled for 5 min in SDS–PAGE sample buffer (50 mM Tris–HCl, 2% SDS, 1% 2-mercaptoethanol, 10% glycerol [pH 6.8]) and electrophoresed through