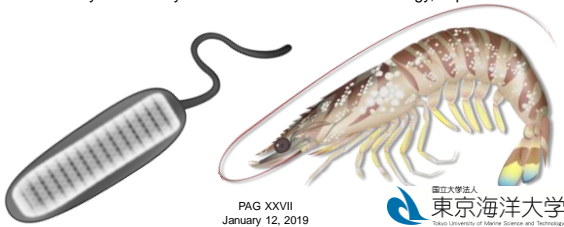
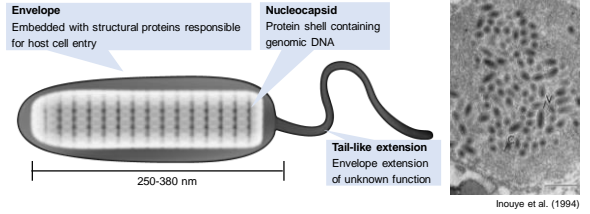


Crustacean Genome Exploration Reveals the Evolutionary Origin of Deadly Shrimp Virus

Satoshi Kawato, Hidehiro Kondo, and Ikuo Hirono
Tokyo University of Marine Science and Technology, Japan

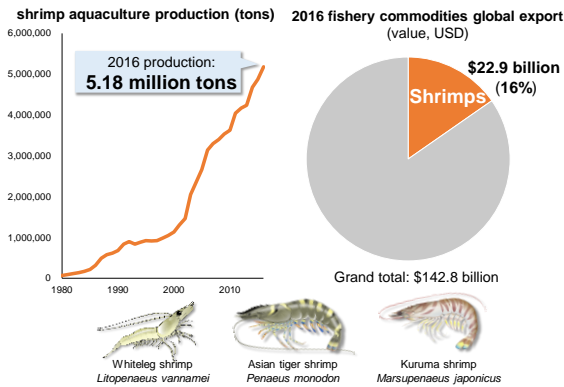


WSSV: enigmatic shrimp virus

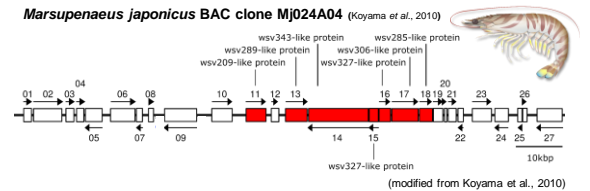


- Double-stranded DNA virus (circular, ca. 300 kbp)
- Extremely broad host range (Lo et al., 1996; Otta et al., 1999)
- Few relatives reported: isolated taxonomic position (family *Nimaviridae*)
- Little knowledge on evolution and phylogeny

Studying shrimp (and their pathogens) does matter



Viral "fossils" buried in the host genome

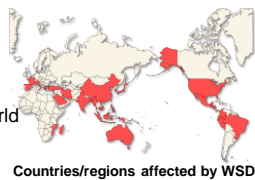


- WSSV homologs in localized in the cell nucleus as repetitive elements (Koyama et al., 2010)
- Over 30 WSSV homologs identified in *M. japonicus* genome (Shitara, unpublished; Wang, unpublished)

White spot disease (WSD)

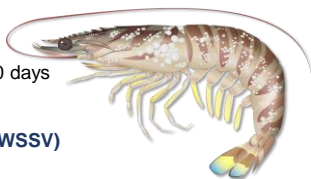
History

1992~: First outbreak in Asia
~2000: \$7 to \$8B estimated loss (Asia and the Americas)
~2010s: Spread throughout the world



Symptoms

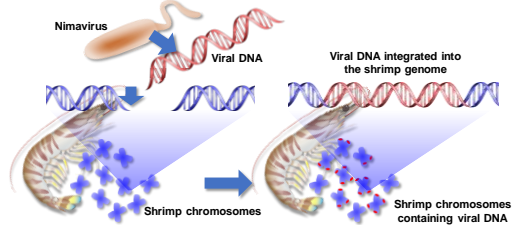
- White spots on the exoskeleton
- Body discoloration
- Lethargy
- 100% mortality within 3-10 days



Causative agent

White spot syndrome virus (WSSV)

Viral "fossils" buried in the host genome

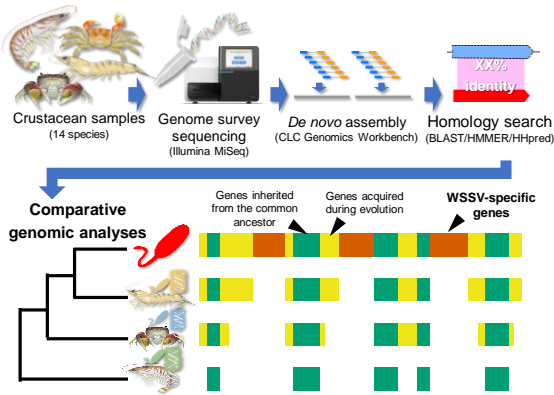


- Crustacean genomes harbor footprints of ancient WSSV relatives (nimaviruses) (Koyama et al., 2010; Huang et al., 2011; Rosenberg et al., 2015)

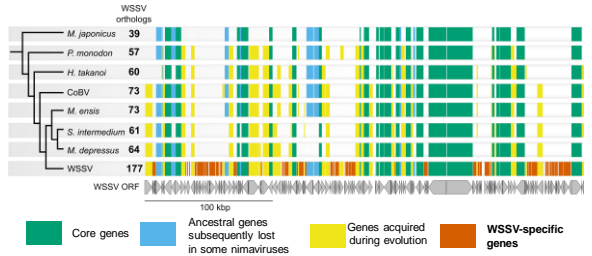
Objective

Comparative genomic analysis of (fossilized) nimaviruses to elucidate the evolutionary history of WSSV

Methods Summary



Comparative genomic analysis of Nimaviridae



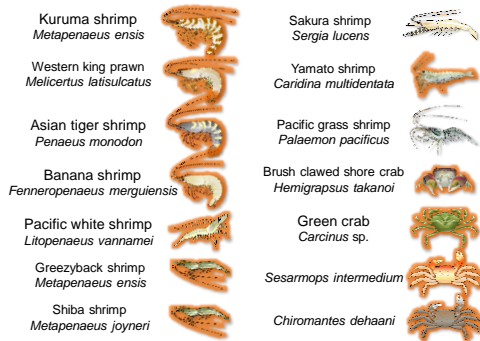
Distribution of WSSV orthologs in Nimaviridae

Predicted genes in the WSSV CN01 genome: **177**

Nimaviral core genes: **28**

WSSV-specific genes: **92**

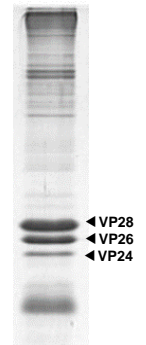
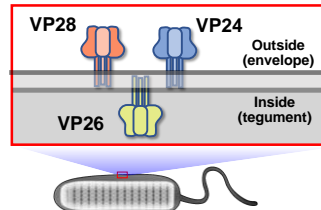
Nimaviral sequences in decapod crustaceans



Nimaviral sequences detected in 12/14 species

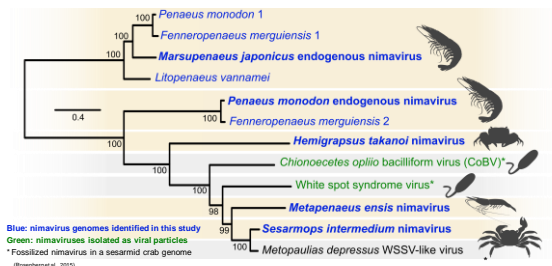
Many WSSV characteristic genes are young genes

WSSV_VP family: the most abundant WSSV structural proteins



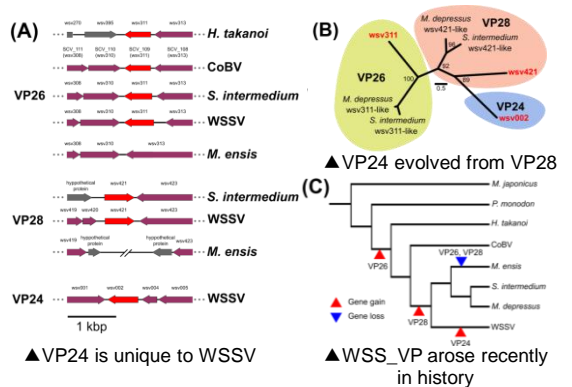
(modified from van Hulten et al., 2000)

Diversity of WSSV relatives



- WSSV relatives of different "genera"
- Diversity and long history of Nimaviridae

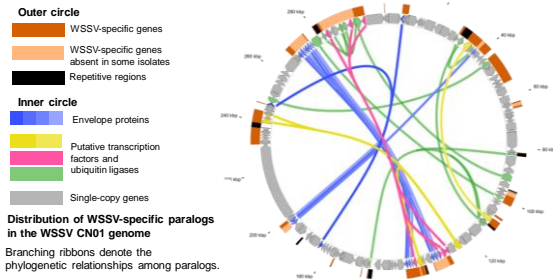
Many WSSV characteristic genes are young genes



▲ VP24 is unique to WSSV

▲ WSSV_VP arose recently in history

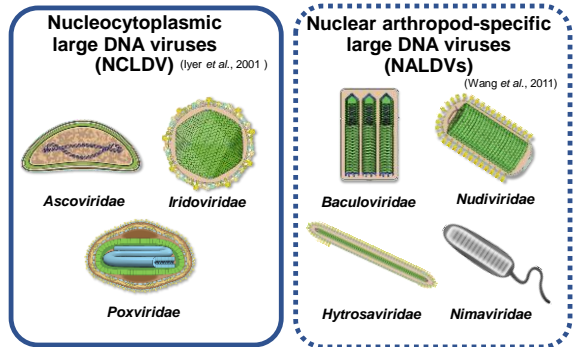
Expansion of host-viral interface by unique accessory gene duplications



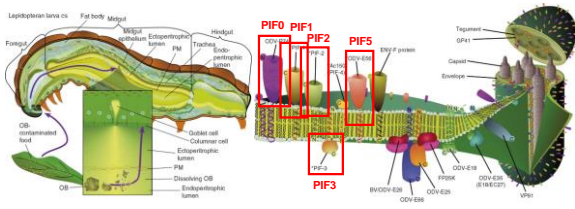
- Unique paralogues accounting for 48% (42/92) of WSSV-specific genes
- WSSV-specific genes: associated with host-viral interactions
- Lacking in some isolates: non-essential accessory genes

Is WSSV a distant offshoot of baculo-like viruses?

Double-stranded DNA viruses infecting arthropods



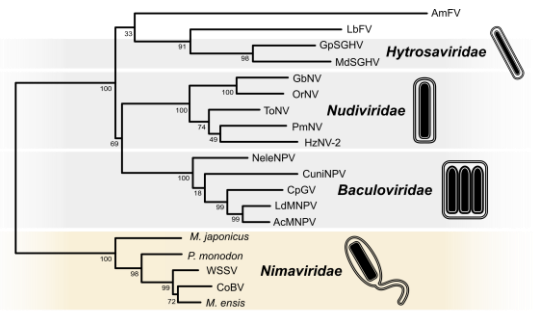
Insect dsDNA virus protein homologs in Nimaviridae



Left: model of baculovirus infection (Slack and Arif, 2006)
Right: PIFs in the baculovirus envelope

Per os infectivity factors (PIFs): a set of envelope proteins conserved among insect DNA viruses, which are required for viral entry into host midgut cells

Is WSSV a distant offshoot of baculo-like viruses?



Maximum likelihood phylogenetic tree of arthropod dsDNA viruses based on seven NALDV core genes

Insect dsDNA virus protein homologs in Nimaviridae

| Function | Name | Baculoviridae | Nudiviridae | Hytrosaviridae | WSSV | Ancestral Nimaviridae | |
|--------------------------------------|----------------------|---------------|-------------|----------------|------|-----------------------|---|
| Transcription (6) | p47 | ● | ● | — | — | — | |
| | lef-8 | ● | ● | — | — | — | |
| | lef-9 | ● | ● | — | — | — | |
| | vif-1 | ● | ● | — | — | — | |
| | lef-4 | ● | ● | — | — | — | |
| | lef-5 | ● | ● | — | — | — | |
| Replication (2) | DNA polymerase | ● | ● | ● | ● | ● | |
| | Helicase | ● | ● | ● | ● | ● | |
| Structural proteins (12) | Oral infectivity (6) | p74 (PIF-0) | ● | ● | ● | ● | ● |
| | | pif-1 | ● | ● | ● | ● | ● |
| | | pif-2 | ● | ● | ● | ● | ● |
| | | pif-3 | ● | ● | ● | ● | ● |
| | | pif-4/19kda | ● | ● | ● | ● | ● |
| | | pif-5/odv-e56 | ● | ● | ● | ● | ● |
| | Morphogenesis (2) | ac68 | ● | ● | — | — | — |
| Unknown (2) | 38K | ● | ● | — | — | — | |
| | vp91/p95 | ● | ● | — | — | — | |
| | vp39 | ● | ● | — | — | — | |
| Others (2) | ac81 | ● | ● | — | — | — | |
| | p33 | ● | ● | — | — | ● | |
| Number of NALDV core gene homologues | | 20 | 20 | 14 | 7 | 7 | |

● Newly identified in this study

(Modified from Wang et al., 2011)

Summary

- Host genome survey revealed previously undetected diversity of WSSV relatives
- Gene duplications enriched WSSV-specific genes involved in host-viral interactions
- Insect dsDNA virus homologs in the ancestral nimavirus gene set suggest a shared phylogenetic origin of arthropod dsDNA viruses

Acknowledgement

- Jumroensri Thawonsuwan
- Hiroyuki Yokooka



Reference

Kawato, S., Shitara, A., Wang, Y., Nozaki, R., Kondo, H., & Hirono, I. (2018). Crustacean Genome Exploration Reveals the Evolutionary Origin of White Spot Syndrome Virus. *Journal of virology*, JVI-01144.

Thank you

Half of the conserved genes are of unknown function

Nimaviral core genes (28)

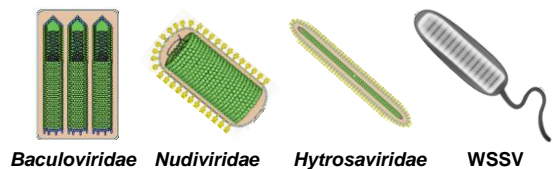
| Function in WSSV | WSSV ORF |
|-------------------------------------|--|
| Structural proteins (11) | |
| Envelope proteins (2) | wsv293a, wsv327 |
| Capsid proteins (7) | wsv037, wsv220, wsv271, wsv289, wsv332, wsv360, wsv415 |
| Unknown (2) | wsv131, wsv161 |
| Non-structural proteins (17) | |
| DNA polymerase | wsv514 |
| Putative TATA binding protein | wsv303 |
| Putative protein kinase | wsv423 |
| Latency-related gene | wsv427 |
| Hypothetical protein (13) | wsv026, wsv133, wsv137, wsv139, wsv192, wsv267, wsv282, wsv285, wsv313, wsv343, wsv433, wsv440, wsv447 |

Marsupenaeus japonicus endogenous nimavirus



- ~220 kbp genome, 2 fragments
- At least 39 WSSV orthologs
- Most distantly related to WSSV
- Homologs present in other 5 species

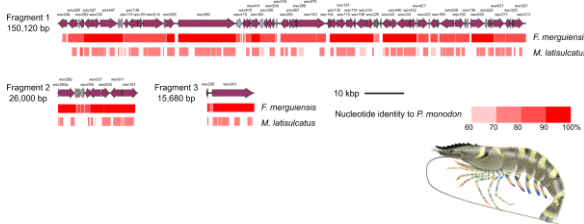
Nuclear arthropod-specific large DNA viruses (NALDV)



- Rod-shaped, enveloped virions
- Circular genomes interspersed with repeat regions
- Replication in the host cell nucleus
- **NALDV core genes**

(Wang et al., 2011)

Penaeus monodon endogenous nimavirus



- ~200 kbp genome, 3 fragments
- At least 39 WSSV orthologs
- Homologs present in 2 other species