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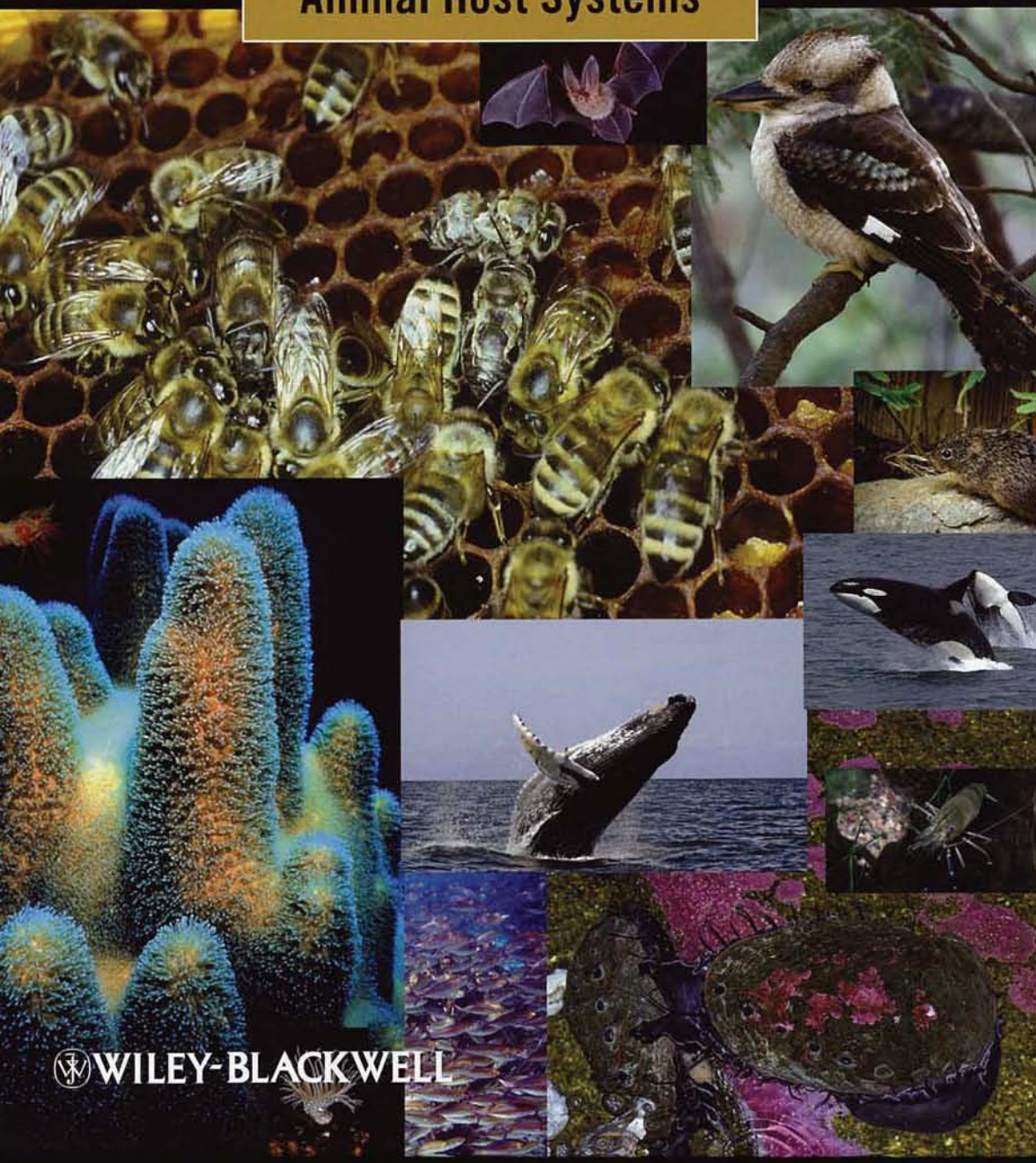
STUDIES IN VIRAL ECOLOGY, VOLUME 2

Animal Host Systems



WILEY-BLACKWELL

CHAPTER 7

THE VIRAL ECOLOGY OF AQUATIC CRUSTACEANS

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7.1 INTRODUCTION AND APPROACH

The ecology of viruses predominately involves the interaction of the virus at the animal and cellular level. When the virions are in the extracellular environment, they are quiescent waiting to infect a living cell. The virions bind to the cell receptors, undergo decapsidation in the phagolysosome, pass the nucleic acid to the cytoplasm or nucleus and begin replication. Evidently, the ecology of viruses starts with entry of the virus into the crustacean, evading the immune system, invasion of the cell, replication, and release of new virions. The crustacean immune system is critical to the functional ecology of their viruses, so we must start with an understanding of this system to have any hope of understanding the ecology of the viruses in crustaceans.

The experimental model for interactions between the crustacean immune system and pathogens has been, by and large, the excellent work by L. Cerenius, K. Soderhall, and coworkers. This model was initially developed on the interplay between the crayfish plague fungi *Aphanomyces astaci* and freshwater crayfish, particularly *Astacus astacus*. Much of the supplementary knowledge on decapod's immunity has come from the true crabs. Unfortunately, the Dendrobranchiata (including penaeids) last shared a common ancestor with the rest of the decapod crustacea during the Silurian epoch, approximately 437 million years ago (Figure 7.1) (Porter et al., 2005). Since that time the immune systems of these lineages have been evolving independently.

Furthermore, the viruses of freshwater crayfish have not been extensively researched because the relatively low economic value of crayfish has not allowed researchers to secure adequate funding. At present, the state of

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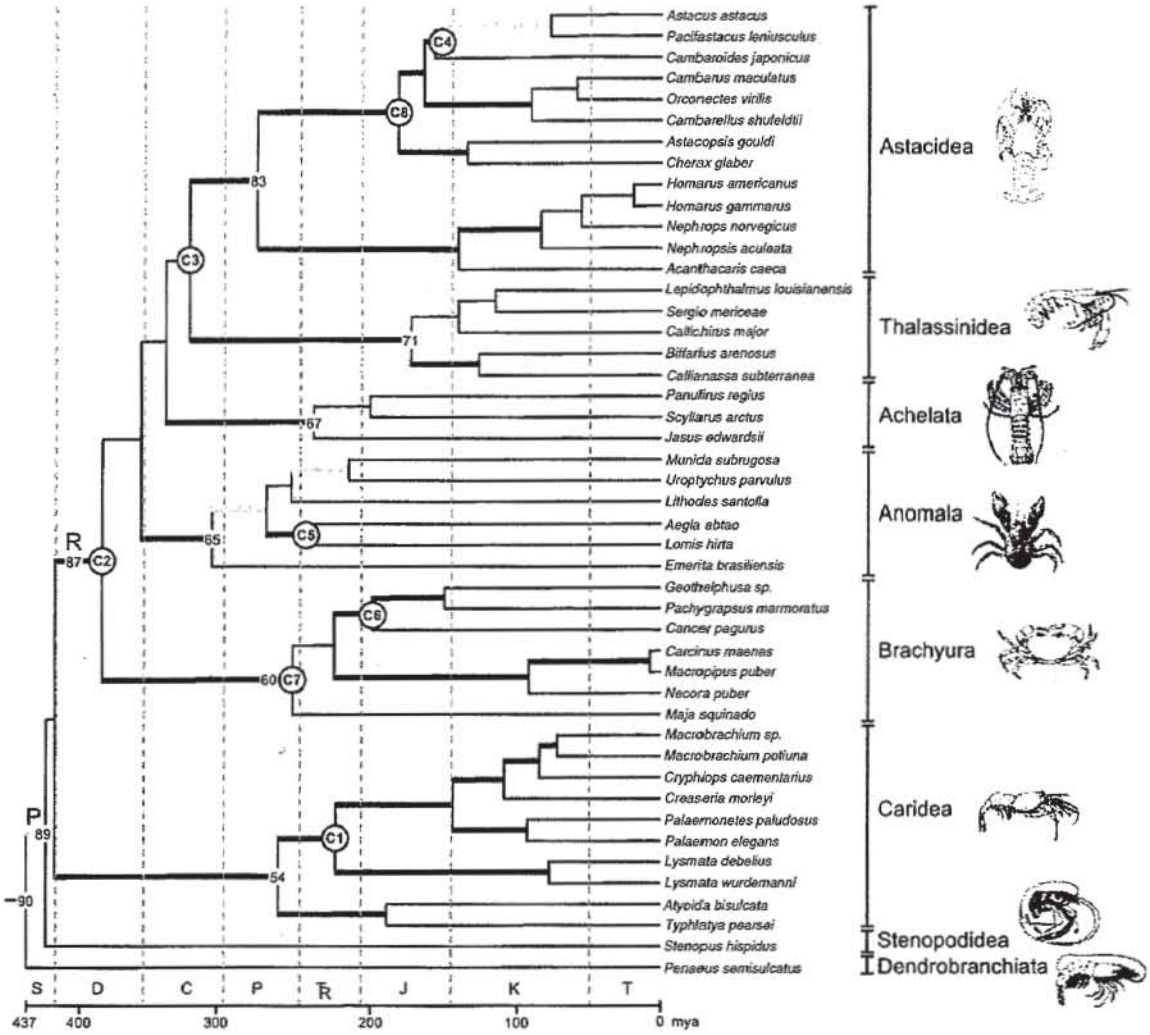

FIGURE 7.1 Decapod divergence time chronogram estimated using topology of ML (maximum likelihood) tree. On branches with both ML bootstrap values of >70% and BMCMC (Bayesian Marker Chain Monte Carlo sampling) $p = P > 0.95$, support is indicated by a thick black line; branches strongly supported by only one tree reconstruction method are indicated by thick gray lines. Fossil calibration nodes are indicated by C1–C8. Node numbers from divergence time estimations are included for reference on nodes of important decapod lineages. The decapod infraorders are delineated, and the nodes corresponding to the suborder Pleocyemata (P) and the informal Reptantia (R) are indicated on the phylogeny. The major geologic periods are also mapped onto the phylogeny, using the following standard symbols: S, Silurian; D, Devonian; C, Carboniferous; P, Permian; Tr, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary. (Adapted from reference Porter et al., 2005.)

knowledge is little more than a catalogue of viruses in hosts and the methods of detection. Therefore, the approach taken in this chapter will be to use the information derived from the penaeids and their interaction with their viruses since the economic power of the aquaculture of penaeids has allowed a much more thorough investigation of their interaction with viruses. Due to the long geological separation of the penaeids from other crustacea, only when information is completely lacking from the penaeids will other comparative information be used.

The taxonomy of the penaeids is very controversial since the premature acceptance of the classification of Perez Farfante and Kensley (1997). Dall (2007) reviewed the controversy based on the morphological and available molecular evidence and largely followed the results of Lavery et al. (2004). He suggested

that it is premature for the promotion of so many subgenera to full genus status. Based mostly on the evidence of Lavery et al. (2004), the facts to date suggests there should only be two genera within the old genus *Penaeus*: *Melicertus* for the old subgenus *Merlicertus* plus *Penaeus japonicus* and *Penaeus* for all other members of the genus. While early and the evidence is still accruing, it is the most up-to-date information available and it will be used in this chapter.

This chapter will not be an updated list of all the viruses found in their crustacean hosts or the methods for detecting the viruses. This approach to the topic has been under taken many times and dealt with in an excellent fashion by international experts such as Lightner (1996) and Flegel (2006) so the reader is referred to their publications for this information.

An alternative approach taken here is to concentrate on the *ecology* of the viruses of crustacea at the subcellular, cellular, and environmental levels.

7.2 THE PENAEID IMMUNE SYSTEM

It is clear that the immune system of decapods has three salient features. One, there is no production of antibodies by B-like cells. Two, the system is characterized by a cascade of cleavage of multiple inactive proteins into the active state by serine proteases. Three, the major triggering compounds are carbohydrates such as peptidoglycan (Gram-positive bacteria), beta 1,3-glucan (fungi), and lipopolysaccharide (LPS) (Gram-negative bacteria). The first of these features needs no further explanation.

The second cascade has been compiled by many authors. The preprophenoloxidase activating (PPA) protein has to be cleaved into an active form by prophenoloxidase activator (a serine protease itself) that cleaves prophenoloxidase into the active phenoloxidase. Phenoloxidase couples with superoxide dismutase that is probably membrane bound on the hemocytes. This turns oxygen-free radicals into hypochlorous acid that oxidizes the microbial invader. Recently, hemocyanin, the oxygen-carrying protein that makes up 95% of the hemolymph protein, has been shown to be cleaved under stress (Cimino et al., 2002) and microbial attack to produce an antimicrobial protein and a phenoloxidase (Lee et al., 2004) which have been shown to be active against a wide range of target microbes.

The triggering of the serine protease cascade by carbohydrates has been elegantly worked out over many years. However, the initial source of the serine protease has not been elucidated. The author hypothesizes that the initial source will be a mannose binding lectin (MBL) pathway. Lectins involved in the immune response in crustacea have been known for many years but their link to other components has not been demonstrated. However, the ability of MBL to be made active by mannose binding in peptidoglycan has been recently demonstrated. MBL in vertebrates has two moieties of two different serine proteases that become active on binding (Figure 7.2). It is hypothesized that the receptors binding to the pattern on mannose residues forces a conformational change to the other end of the lectin where the serine proteases are situated and they become active. This is an elegant way of activating the many cleaving enzymes of the immune system right at the surface of the microbe that needs to be destroyed.

The implications of understanding the functioning of the immune system in penaeids are astonishing. First of all, because the immune system is carbohydrate based rather than protein based as in the vertebrate lineages, the crustacean immune system did not evolve to deal with viruses that have limited, if any, carbohydrate moieties on their surface and where carbohydrates do occur they are not in the pattern necessary to change the conformational shape of the MBL-linked serine protease to activate them. Therefore, crustaceans have had to develop a second, totally independent immune system to deal with viruses separately.

Second, there is an implication that modern viruses of eukaryotes evolved after the

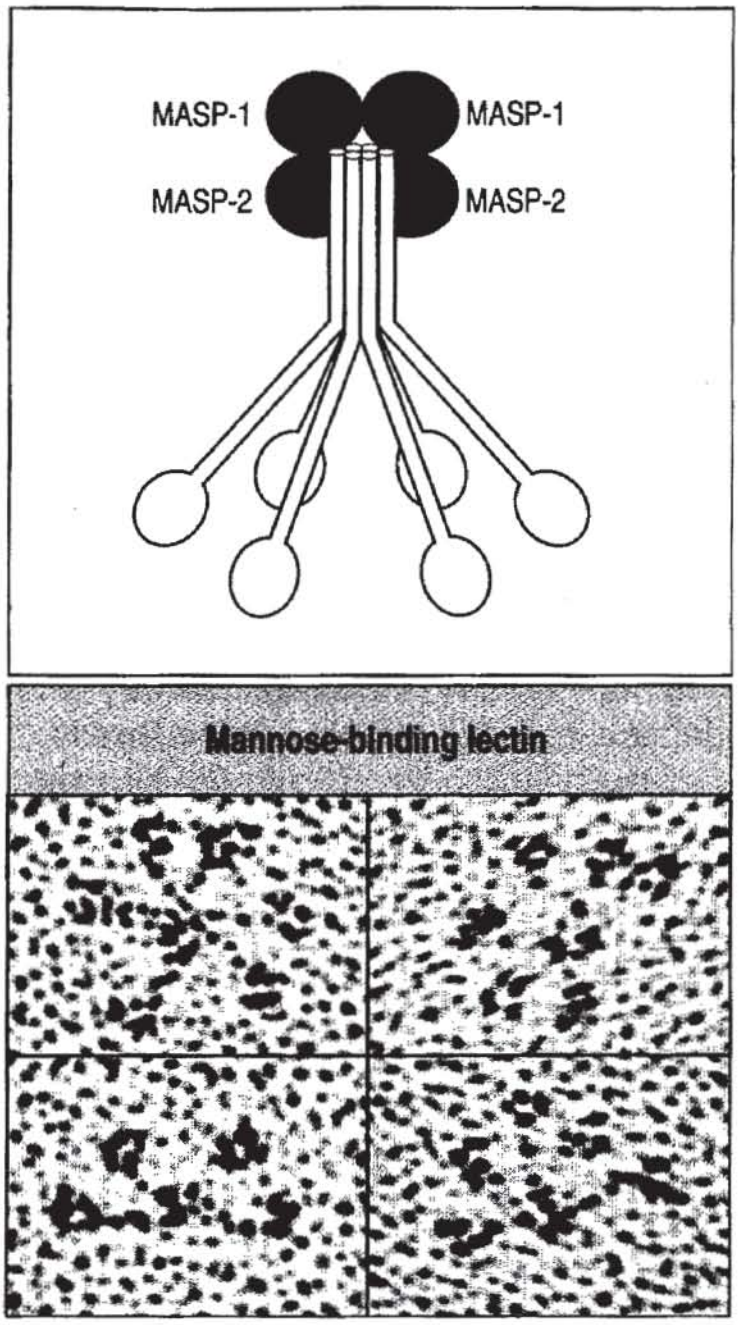

FIGURE 7.2 A schematic and electron micrograph of a mannose binding lectin from mammals that resembles the complement C1 complex. Mannose binding lectin forms clusters of two to six carbohydrate binding heads around a collagen-like stalk. This structure is discernable under the electron microscope (lower panels) (photograph courtesy of K. B. M. Reid). Associated with this complex are two serine proteases, MBL-associated serine protease 1 (MASP-1) and 2 (MASP-2). (Adapted from reference Janeway et al., 2005.)

crustaceans. Otherwise, it is likely that the antiviral immunity would have clear roots to the antipathogen system that was already there, that is, some use of the prophenoloxidase cascade to destroy viruses.

7.2.1 The Theory of Viral Accommodation

The theory of accommodation of viruses in crustacea has been championed by a series of publications by Flegel and his coworkers

(Flegel and Pasharawipas, 1998; Flegel, 2007; Flegel, 2009). In short, the viral accommodation theory suggests that after some generations, crustaceans tolerate viruses by locking the virus away in infected cells. The host stops the cells from lysing, preventing the release of virions or destroying large amount of host tissues. The theory grew out of a number of field observations. First, there was the lack of a vertebrate inflammation-like response around tissues which clearly showed viral inclusion bodies (Flegel and Pasharawipas, 1998). Second, there was the observation that after 2 or 3 years of epizootic mortalities in penaeids when a new virus was introduced to näve populations, mortalities decreased to lower background levels even though the animals were demonstrably persistently infected with the virus (Flegel and Pasharawipas, 1998). Penaeids survived and grew to reproductive age, but they were more susceptible to environmental perturbations that could trigger mortality events. The control of apoptosis of viral infected cells was suggested as the mechanism that restricted mortality. However with excessive environmental fluctuations, the penaeids lost their ability to control apoptosis leading to systemic widespread cell death and subsequent animal mortality. The role of apoptosis in viral induced mortality is controversial and therefore not universally accepted (see Flegel, 2007). Nevertheless data from Midcrop Mortality Syndrome in *Penaeus monodon* from Australia supports the theory (Anggraeni and Owens, 2000).

One of the implications of the accommodation theory is that the tolerance to the virus must be passed on in a heritable manner so that the next one or two generations can also become tolerant to the virus. In recent years, it has been established that the interfering RNA (iRNA) pathway exists in all eukaryotes that have been investigated including crustacea and terrestrial crustacea (e.g., insects (see Regier et al., 2010)). Within the iRNA pathway, there are two separate components that operate a nonspecific dsRNA knockdown where the presence of any dsRNA enhances the RNA-induced silencing complex (RISC) and a specific dsRNA knockdown that is much more efficient at downregulating a viral gene (Robalino et al., 2004, 2005, 2007; La Fauce and Owens, 2009). Nonspecific dsRNA silencing maybe why prior infections with some viruses leads to some cross-protection against other viruses. For example, infection with infectious hypodermal and hematopoietic necrosis virus (IHHNV, *Penaeus stylirostris* densovirus, genus *Brevidensovirus*, family Parvoviridae, Figure 7.3) has been found to give protection to subsequent infection with white spot syndrome virus (WSSV, genus *Whispovirus*, family Nimaviridae, Figure 7.4) (Melena et al., 2006).

Flegel (2009) has used the presence of iRNA in crustacea to propose a mode of action for viral accommodation. It is proposed that nucleases, reverse transcriptase, and integrases that are common in the crustacean genome chop up the viral genome, convert RNA into DNA, and integrate the DNA into the crustacean genome as small fragments of viral ghost DNA. These subsequently act as template for iRNA pathway to knockdown mRNA of viral genes thus reducing the viral load below a critical threshold level that would cause disease. Through natural selection, those surviving animals having nondeleterious and beneficial inserts corresponding to viral ghost DNA contribute rapidly to the gene pool for the next generation leading to widespread tolerance.

There is some evidence that might suggest a mechanism of how the viral ghost DNA gets into the next generation's germ line. When crustacean hemocytes are not combating pathogens, they have a secondary function of shuttling lipoproteins from the hepatopancreas to the ovary (Dr. Ester Lubzens, National Institute of Oceanography, Israel, personal communication). The high-density lipoprotein 1 of *Penaeus semisulcatus* is a homologue of beta 1,3-glucan binding protein found in hemocytes, which probably binds to an ovarian lipoprotein receptor. If the hemocytes have phagocytosed virions and processed them to a short DNA structure in phagolysosomes, this would be a

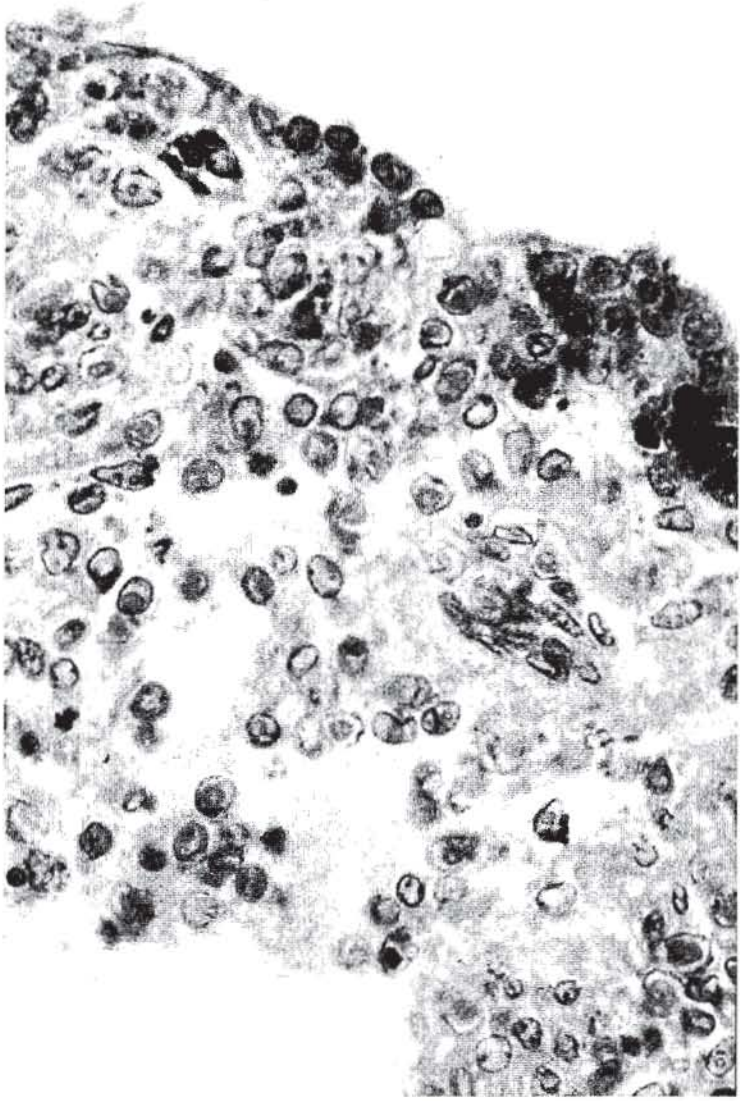

FIGURE 7.3 Infectious hypodermal and hematopoietic necrosis virus (IHHNV, *P. stylirostris* densovirus) infecting the lymphoid organ of a hybrid *P. monodon* crossed with *P. esculentus*. Note almost every cell has an eosinophilic Cowdrey A intranuclear inclusion body. (*See the color version of this figure in Color Plates section.*)

perfect way to shuttle the viral ghost DNA into the germ cells of the next generation as it shuttles the lipoprotein into the eggs. It also opens the door to inappropriately processed virus, that is, live virus also being shuttled into gametes.

Once a hemocyte has exocytosed its active components, then phagocytosed virions either have their nucleic acid processed or not as the case may be and then passed viral ghost template to the germ cells, there remains the problem of what to do with the spent hemocytes. If viral processing is not complete, then destruction of the hemocytes runs the risk of liberating unprocessed infectious virus or infectious nucleic acid such as with TSV (Taura syndrome virus, family Dicistroviridae, genus unassigned). So the penaeids sequester the spent hemocytes into the lymphoid organ to produce spheroids that lock the virus away out of circulation (Anggraeni and Owens, 2000, Figure 7.5). These spheroids can contain live infectious virus for some considerable time (Hasson et al., 1999), but spheroids eventually get encapsulated and disposed off via an unknown mechanism perhaps harmonized to the lunar cycle (Rusaini and Owens, 2010a). This is why so many viruses have been found

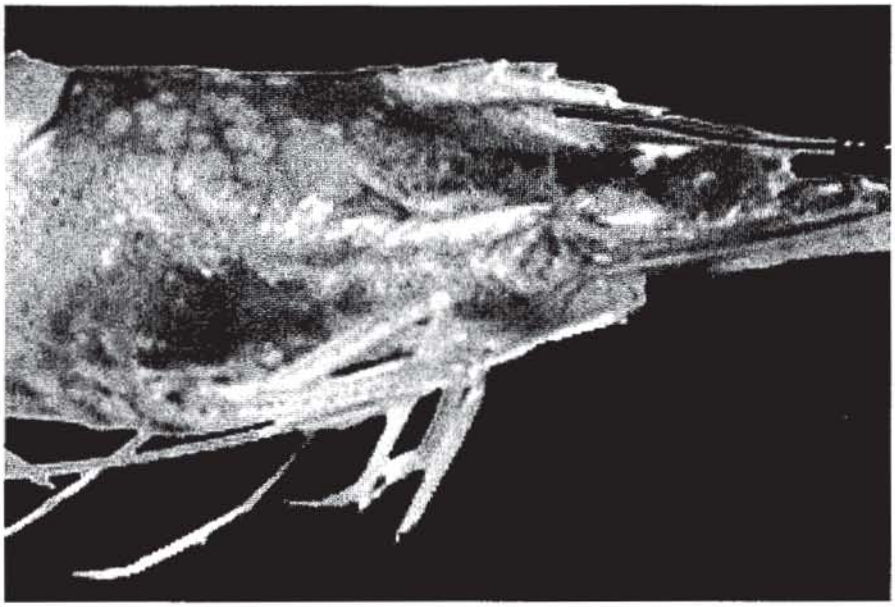

FIGURE 7.4 Clinical signs of white spot syndrome virus in *P. merguiensis*. Note the white lesions on the rear of the cephalothorax. Photograph by K. Claydon and L. Owens. (*See the color version of this figure in Color Plates section.*)

located in the lymphoid organs (Rusaini and Owens, 2010b). As the lymphoid organ is not found outside the penaeids, other tissues or individual spent hemocytes must be doing an equivalent role in other crustacea.

The theory on the accommodation of viruses leading to a heritable tolerance has now been tested both experimentally and by natural epizootics. While not all components are understood, it is proving to be a very robust and

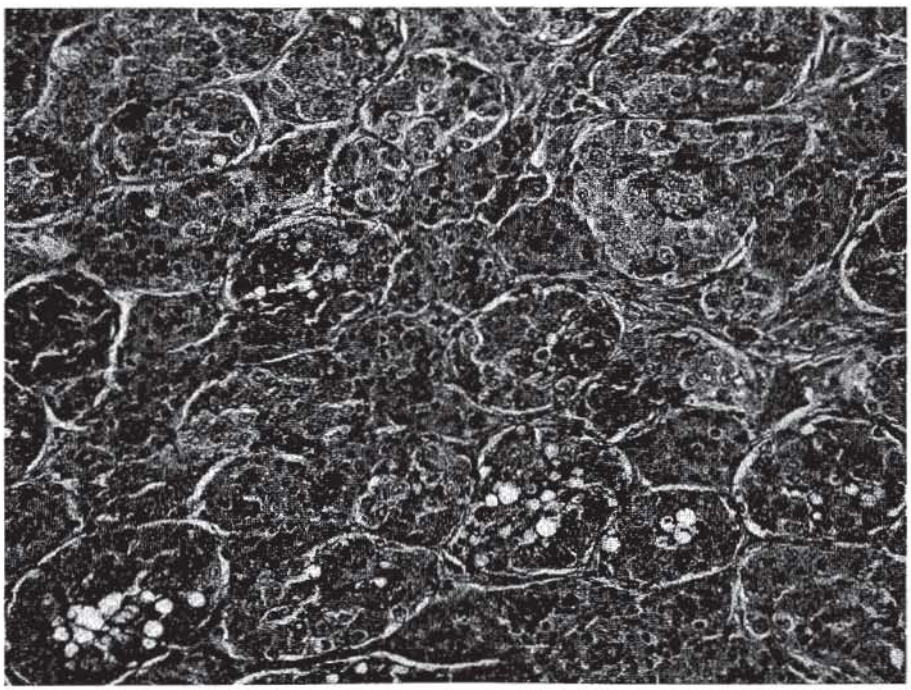

FIGURE 7.5 Lymphoid spheroids in the lymphoid organ of *P. merguiensis*. The round, more basophilic sections are the spheroids made up of spent hemocytes and the more eosinophilic tissue with the central hemolymph vessels are the normal stromal matrix areas of the lymphoid organ. (*See the color version of this figure in Color Plates section.*)

useful tool for understanding the dynamics of viral infections and tolerance of infections in crustacea.

7.3 THE VIRUSES FIGHT BACK

Viruses have evolved to side step some of the mechanisms that the crustaceans use to defeat them. At this stage, it is unclear if the viruses evolved these mechanisms in crustacea or having these mechanisms has allowed the viruses to infect crustacea more efficiently. Crustacean baculoviruses have the inhibition of apoptosis (IAP) genes, P35/38. The dicistrovirus, Taura syndrome virus has a biochemical ability similar to the baculoviral inhibitor of apoptosis protein repeat (BIR) gene (Mari et al., 2002). Both of these genes have the ability to stop the early apoptotic destruction of infected cells allowing full replication of the virus. These genes are transcribed in the immediate early phase of viral genome transcription ensuring they are functioning very early in the infection process. This is critical in the case of baculoviruses that must produce a massive polyhedral protein. The baculovirus cannot have the infected cell lysed early or the polyhedral protein will not be produced to protect the virions in the harsh intertidal zone at low tide from the ravages of UV light, desiccation, and high temperature.

Macrobrachium rosenbergii nodavirus (an unclassified member of the family Nodaviridae) produces the B2 protein from viral genome segment 1 that binds at multiple sites to the dsRNA intermediates of the virus. This then prevents the *Dicer* enzyme of the crustacean iRNA pathway from being able to cut up intermediates and therefore silence the virus.

The studying and understanding of the genes of crustacean viruses is in its infancy and severely hampered by the lack of crustacean cell lines that would allow manipulation of the viruses. As viral sequencing and classification of genes proceeds, it is anticipated that more information on how the viruses evade the host responses as well as the mechanism that some already discovered systems (i.e., IAP) genes function will be unveiled.

7.4 WHERE DO VIRUSES COME FROM?

Where does the crustacean index case that starts a viral epizootic get its infective load from? I believe most new viruses come from the practice of feeding rich maturation diets to broodstock. Broodstock need high levels of protein and particularly lipids to produce healthy eggs and larvae. Aquaculture has developed a number of maturation diets that include in particular, marine invertebrates. One practice that became particularly common before the worldwide WSSV outbreak was to feed frozen crabs broken up with a hammer, directly to the broodstock. This may explain why most of the other possible species of the genus *Whispovirus* (the genus of viruses that WSSV belongs to) are all found in crabs. There has been a tendency of late to restrict the feeding of crustacea to crustacean broodstock and a concomitant move toward the use of other invertebrates such as polychaetes, bivalves, and cephalopods as maturation feeds. Recently, the rate of emergence of new catastrophic viruses in crustaceans has seemed to have slowed and hopefully the two are related as a cause and beneficial effect of changing dietary feeding practices.

7.4.1 Local Spread of Viruses

Once the broodstock are infected, it is only a matter of time before the larvae become infected. Female broodstock defecate just prior to spawning. The eggs are then broadcast spawned into this milieu of enteric viruses, bacteria, and undigested food. The Tahitian method of separating contaminated egg shells and weaker larvae from healthy larvae has been instrumental in the decline in the importance as pathogens of baculoviruses, unassigned rod-shaped viruses, and perhaps the enteric densoviruses.

With the systematic viruses it is more difficult to prove the mode of infection. However, the huge successes using PCR-testing of broodstock or postlarvae (the postplanktonic stage of penaeids) and only stocking with viral free stock or lightly infected postlarvae has shown the power of the broodstock link. Perhaps systemic viruses are shed on spawning as the eggs are expelled, but one would expect the Tahitian method (see above) and surface sterilizing of the eggs would have reduced the impact of these systemic viruses. However, this does not seem to be the case. Therefore, it seems likely that the virions are shuttled to the ovaries with the lipids (see above) and are under the vitelline membranes when spawned.

Once a viral epizootic is underway it is easy for virions to transmit to the next host as densities of hosts in aquaculture are among the highest in any animal production system. With WSSV in experimental situations, waterborne virion loads have been shown to be sufficient to transmit disease to animals sharing only the same water. Unfortunately, many of the studies investigating waterborne infections were flawed in that the method of separating infected crustacea from animals being exposed to the water would not have stopped small pieces of pleopods, pereiopods, uropods, gills, antennae, and shredded tissue produced during cannibalization from being washed into the trial tanks where they could be consumed. While the concept of viruses being waterborne does seem logical for enteric viruses such as the baculoviruses and densoviruses, it does seem counterintuitive for those viruses that are systemic such as WSSV, yellow head virus (genus *Okavirus*, family Roniviridae), gill-associated virus (genus *Okavirus*, family Roniviridae, Figure 7.6), infectious myonecrosis virus (IMNV, presumably an unclassified member of the viral family Totiviridae) and IHHNV. So then we must ask, "How do the systemic virions escape the carcasses?" It is probable that during cannibalization the tissue is shredded enough to release virions.

There is no doubt that cannibalization is the main method of viral spread once an epizootic is underway. The most predatory species (e.g., *P. monodon*, *M. japonicus*) suffer more acute viral diseases, higher mortality, and suffer more viral diseases than other species. This has partially led to decreased tonnage of these species being recorded by farmers as they move away from these more problematic species.

Within the literature, there are a large number of publications that have identified animals living in the aquatic environments as alternate

FIGURE 7.6 *P. monodon* infected with gill-associated virus. Note the yellow gills similar to symptoms of prawns infected with the conspecific yellow head virus. (*See the color version of this figure in Color Plates section.*)

hosts or carriers for crustacean viruses. Unfortunately, there has not been a robust set of criteria applied to the generation of these lists. Most studies have used PCR-based detection that cannot tell if the virion is infectious or whether the PCR is amplifying ghost DNA. No confirmatory sequencing or infection studies were described in the majority of these studies. We have seen above how nucleases, *Dicer*, RISC, reverse transcriptase, and integrases process RNA into a DNA signature in the genome. Furthermore, other research in terrestrial crustacean cell lines, the mosquito cell line *Aedes albopictus* C6/36, has demonstrated the rapid accumulation of persistent interfering particles (PIP) from 3% in the original viral inoculum to 30% over four generations (Roekring et al., 2006). These persistent interfering particles were shown to be highly unlikely to be infectious due to frame shifts in the viral genome that encode for critical proteins. PIP may be empirical evidence of another method that crustaceans use to deal with viruses, or it might be an unknown precursor in the iRNA pathway or most likely, errors in matching the crustacean's cellular replication machinery with the viral transcripts. It is likely that the more unsuitable a host cell is for a virus, the more probable transcription errors will occur. This fact is used by vaccine manufacturers to produce mutant, weakened viruses for vaccine candidates. All of these modifications to viral genomes could give PCR signals of appropriate sizes but the virions are not, in fact, infectious. Therefore, many of the lists of alternate hosts for viruses are very suspect without confirmatory studies of some kind.

7.4.2 Geographical Spread of Viruses

The way that viruses spread from country to country, continent to continent, and from ocean to ocean is hotly debated as no country wants someone else's viral problem. Furthermore, some jurisdictions have viewed the threat to their own industries to be sufficiently severe as to apply restrictive conditions under the International Zoo-sanitary Code, which increases the level of global tensions. Nevertheless, it is impossible to argue that any process that allows crustaceans to arrive alive or in an unprocessed state fit for human consumption will not carry viable virions if they were present in the animals when harvested. The evidence is overwhelming that moving contaminated live broodstock or postlarvae has been responsible for transcontinental movement of crustacean viruses. The negligent movement of "clean" but untested broodstock or postlarvae is believed to have been responsible for the introduction of IHHNV and TSV into Hawaii, IHHNV into Mexico, IMNV into Indonesia, and Gill-associated virus into SE Asia. Lightner (1990) published a figure showing the then-known movement of live penaeids demonstrating the effectiveness of the "jumbo jet vector" in moving live crustaceans around the globe.

In every case tested experimentally, the viruses detected in frozen commodity shrimp were viable and caused disease and mortality in indicator crustaceans (e.g., Nunan et al., 1998; McColl et al., 2004). Processing of frozen commodity shrimp has been implicated in the transfer of WSSV to the American continents. Furthermore, birds have been implicated in the spread of viruses from rubbish dumps containing commodity shrimp wastes. This hypothesis has received support from studies that show that viable, nonenveloped viruses can pass through the gut of seagulls (Garza et al., 1997; Vanpatten et al., 2004) and chickens (Vanpatten et al., 2004).

7.5 ORPHAN VIRUSES IN CRUSTACEA?

Orphan viruses are those viruses found in a host but are not considered to cause any disease. While orphan viruses are believed to exist in crustacea, as evidence accrues it appears less and less likely that this is the case. The first piece of evidence was from hepatopancreatic parvovirus (HPV; taxonomic name, *P. monodon* densovirus). Apart from the original paper on HPV's discovery in wild *Penaeus merguiensis*

and *Penaeus indicus* from Singapore (Chong and Loh, 1984), and in general review articles (e.g., Lightner, 1996) that attributed up to 100% mortalities during outbreaks, this virus was largely ignored because the industry believed it did not impact on production. However, Flegel et al. (1999) demonstrated statistically significant stunting caused by HPV. Recently Owens et al. (unpublished) have demonstrated a statistically significant 28% loss of production in *P. merguiensis* production due to a sister virus *P. merguiensis* densovirus.

Australian freshwater crayfish have in their hepatopancreas three so-called orphan viruses, Cherax intranuclear bacilliform virus (possibly an unclassified member of the viral family Baculoviridae), Cherax giardiavirus-like virus (presumably an unclassified member of the genus *Giardiavirus*, family Totiviridae), and Cherax reovirus (presumably an unclassified member of the family Reoviridae) that have been discounted by industry as unimportant. However, when hatchery technology was developed that allowed eggs to be surface sterilized thus producing eggs-specific pathogen free for these viruses, average size at harvest went from 35 to 70 g and the production cycle was shortened by 1 month.

If you consider that any virus must be at the very least removing cells from their normal function, upregulating immune functioning cells and diverting energy for nucleic acid processing (see above), then clearly there must always be a metabolic cost to any viral infection. There are no true orphan viruses in crustacea. However, whether it is economical to remove a virus from a growing system is another question.

7.6 CONCLUSIONS

To comprehend the ecology of viruses in aquatic crustacea, it is necessary to appreciate the immune system of crustacea because you cannot understand one without the other. Furthermore, most reader's backgrounds in immunology are from a mammalian viewpoint that does not necessarily set the scene for immediate understanding to the interrelationship of crustaceans and their viruses. As the main crustacean immune response is carbohydrate based, then crustaceans have taken on a different strategy for dealing with proteinaceous viruses. The steps of their strategy include isolating the viruses in cells, preventing the infected cells from being destroyed (control of apoptosis) thus not releasing progeny virions, passing memory iRNA molecules to germ cells, locking up infected hemocytes in lymphoid spheroid cells where the associated viruses can do no harm and then, breeding before either the internal containment system collapses via uncontrolled apoptosis induced by environmental fluctuations or predation kills the crustacean. Fundamentally, this has meant that most virus-exposed survivors are chronic carriers of the virus for life.

With the understanding of the antiviral immune system in crustacea, it is no longer correct to state that crustaceans do not have acquired immunity or immune memory as both of these operate within the iRNA and viral accommodation systems.

Broodstock practices and the global transport of shrimp as live and frozen commodities have been instrumental in the spread of viruses globally. Only the use of animals that truly are free of pathogens, coupled with attention to biosecurity, can bring back the heady days when crustacean aquaculture was rapidly approaching an industry generating US$ 20 billion per year. Intertwined with this is the necessity of a change in industry's attitude toward investment in antidisease research to take it from a "fire fighting exercise" to a progressive, structured program. This is more imperative than ever as research has demonstrated a very low heritability for resistance to viruses such as WSSV (e.g., Gitterle et al., 2005). Many researchers and farmers have promulgated that genetic selection was the answer to all the problems with viruses.

There is a need for a critical review of all the publications that list new crustacean hosts for viruses that have relied on only PCR as

evidence of infection. The minimum methodology for any new publications in this area must be PCR signal plus a confirmation test that could include experimental exposure in a susceptible host or mRNA signal confirmed by sequencing or probing to show transcription of viral genes. In time as knowledge of ghost viral signatures increases, a multiple locus sequence analysis might be possible.

The future potentially includes the ability to transfect crustaceans with genes that can upregulate desirable abilities and transfect with nucleic acid constructs including iRNA that can downregulate viral proteins, that is, further blocking of the apoptosis cascade or the incorporation of betaine genes into the genome might be useful in situations of environmental stress. However, society's fear of genetically modified organisms, that is, "Frankenshrimp" will have to be overcome first or there will be a limited market for an expensive-to-produce commodity.

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