Master scriptie Evolutionary Ecology
21-08-2008

Insects and their viruses

or: viruses and their insect hosts

by Fardo Witsenburg

supervised by Louis van de Zande

Table of contents

Abstract	**2**
Introduction	**3**
The insect innate immunity against viruses	**5**
Viral evasion of the immune system and resulting arms race	**8**
Coevolution	**9**
Life-history	**11**
The ecological role of viruses and their insects	**13**
Synthesis	**15**
References	**18**

Abstract

Viruses are omnipresent and form a threat to all life forms. How insects deal with viruses remains relatively unknown, compared with fungi and bacteria. This paper gives an overview of insect-virus interactions on different levels. How does the insect immune system fight off virus infections? Can insects influence virulence? What is the ecological significance of insect viruses?

Three anti-viral immune responses have been identified up to now. The Jak-STAT pathway is activated in response to viral presence. RNA interference can block virus replication by destroying viral genomes. Cell apoptosis destroys entire infected cells. Viruses use suppressors of RNAi and apoptosis inhibitors as counter measures. This arms race, in which every measure demands a counter measure of the other party, results in coevolution of the two species.

As the virus successfully infects the insect it will generally shorten the insect's lifespan and reduce its fecundity, though there are exceptions. The prevalence of a virus infection on the other hand is dependent on the success of its mode of transmission, which can be manipulated by insects. If the virus is successful in its transmission and replication it can dramatically diminish an insect population. Population dynamics are not dictated by virus prevalence but are under their influence. In insect ecology a virus can function as a control of insect density, as a symbiotic weapon against other insects and as a passenger transported to other hosts. The insect-virus system is a good model system for studying disease dynamics and has potential for pest control.

Introduction

If one wants to study the response of honey bees to a certain virus, say bee virus X (BVX), a simple experimental strategy would be to take healthy bees and inject them with a solution containing BVX. To separate the effect of injecting solution from the actual virus infection, a control group of healthy bees should be injected with pure water. After a few days the virus infected bees will show some form of pathology, specific to BVX. But amazingly, the healthy bees injected with nothing more than sterile water, will in the mean time have developed a wide variety of pathologies, characteristic to several other viruses.

Anderson and Gibbs (1988) looked into this phenomenon and could confirm that the water-injected honey bees (*Apis mellifera*) indeed had grown sick by one of at least 4 different viruses. They ruled out the process of injection as the cause of infection. This led Anderson and Gibbs to the conclusion that the virus must have already been present in the bee, albeit in an inactive state. Furthermore, because of the high incidence of the viruses, most bees should have been infected with several different viral species. Yet in every bee only one virus had replicated to pathological and immuno-essay detectable amounts. Apparently the replicating virus suppresses the others (Anderson & Gibbs 1988).

The experiment above illustrates how counter-intuitive antiviral responses of insects can be. Insects can live happily while carrying several possibly lethal viruses. Viruses can from one day to another change their virulence, and prevent others from doing the same. These behaviours ask for an explanation. And for consequences. In case of the bees, one of their evolutionary responses was to mate polyandrously. Multiple mated queens have less virus prevalence in their hives, produce more offspring and have an increased hive's comb size (Tarpy & Seeley 2006). Polyandrous queen bees therefore facilitate a higher honey production.

But the honey bee has a bigger economic importance than producing sweets. Considering their pollinating function for many agricultural crops, the total economic value of *Apis mellifera* is estimated in the tens of billions of dollar. The contribution to the world economy of the entire insect population is of course invaluable. In ecology, insects play a key role in many processes. Evolutionary speaking these roles are not irreplaceable but in contemporary ecological contexts, a sudden virus-induced eradication of an insect population would not remain unnoticed by the rest of the system.

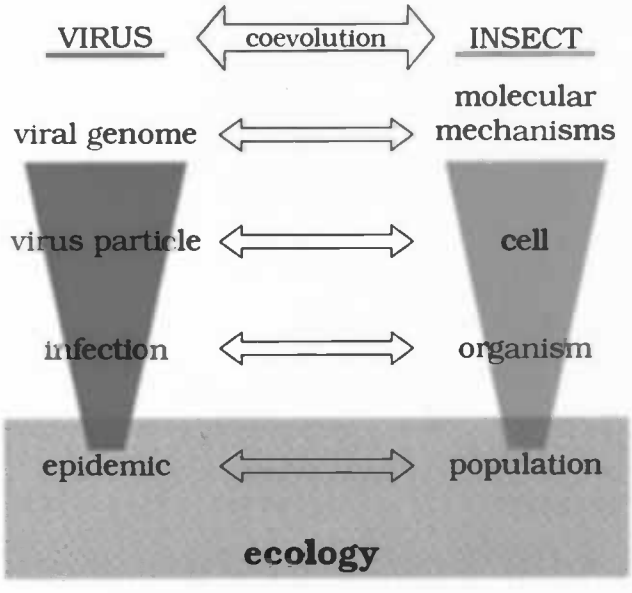

figure 1. An overview of interactions between insects and viruses on different scales. All interactions together will lead to coevolution of the two involved species.

40% of *Drosophila* flies carry with them horizontally acquired viruses; another 20% is virus-infected by inheritance from their parents (Lemaitre & Hoffmann 2007). Compared to bacterial and fungal resistance of insects, only little is known of how insects cope with viruses. Insects lack the vertebrate acquired immune system and therefore have to counter virus attacks using only inherited mechanisms. The earlier described bee-experiment illustrates how different the resulting interactions between virus and insect can be, compared to our well known vertebrate system.

Viruses and insects are both familiar species, to each other and to science. On how these two influence each other much less is clear. In this paper I will give a broad overview of the interactions between virus and insect. These interactions can occur on different levels, from molecular to ecological, and these different levels of interaction are used to broadly structure this paper.

The first chapter describes our current knowledge of the insect innate immune system; the molecular and cellular weaponry it has available against virus invasions. The second chapter takes the perspective of the virus of how it can evade these cellular strategies of the host. The resulting arms race between virus and host will lead to coevolution of the two species. This process is clarified in chapter 3. The fourth chapter looks at an organismal level how virus infections influence the insect's life-history and vice versa; how insects may influence the success of an infection. Of course the insect host and its invading virus are only two actors in a bigger context. The fifth chapter puts the insect-virus interactions in an ecological context and shows briefly how populations and third parties are affected by, and contribute to these dynamics. The end chapter integrates the previous chapters and shows how current and future knowledge of insect-virus interactions can be applied in practice.

The insect innate immunity against viruses

Most of what we know about the mechanisms of insect immunity has been based on studies on *Drosophila melanogaster.* The innate immune system has a cellular facet, which encompasses cellular phagocytosis of the microbes or local encapsulation of larger metazoans by plasmatocytes (Schmidt *et al.* 2001) and a humoral facet (Hoffmann 1995); the insect fat body, salivary glands, gut cells and cells in the genital tract can produce antimicrobial peptides (Hoffmann *et al.* 1996). These peptides target specific types of microbial intruders. The production of these specific peptides depends on one of two pathways. Gram+ bacteria and fungi induce the Toll pathway, Gram– bacteria trigger the Imd pathway (Hoffmann 2003). Larger intruders (e.g. eggs and larvae of endoparasitic wasps) are recognized through the glycoproteins in their basement membrane by hemocytes. This starts a cascade leading to the encapsulation and melanization of the non-self tissue (Schmidt *et al.* 2001).

The viral immunity response does not seem to use any of the above mentioned pathways. In fact, while the antifungal and antibacterial reactions of insects have been tracked in detail, the protecting mechanisms against viruses remained obscure for a long time (Clem 2001). A recent study demonstrated the first pathway involved in countering virus infections; the Jak-STAT pathway (Dostert *et al.* 2005). In insects this pathway has all its known functions in embryonic segmentation and other aspects of development (Dostert *et al.* 2005). In mammals on the other hand, the Jak-STAT pathway's involvement in the antiviral response has been known for a long time (Lemaitre & Hoffmann 2007). It appears that insects share this function.

Exposed to Drosophila C virus (DCV), cultured drosophila cells up regulated 140 genes, which have in their 5' upstream sequence the binding site for a STAT transcription factor and require Jak-kinase for transcription. Two thirds of the genes involved are not induced by bacterial of fungal infections. One third these genes have unknown functions. The most interesting among these is *vir-1.* This gene gets expressed in cells which themselves have not (yet) been infected by the current virus (Dostert *et al.* 2005). This suggests that these cells react to some toxic by products of the infection or cytokines released as a signal by the infected cells (Cherry & Silverman 2006). The Jak-STAT pathway alone was not enough to express *vir-1*; its promotor most likely integrates this pathway with another, unknown signalling pathway (Dostert *et al.* 2005).

How the cell recognizes the virus infection and subsequently activates the Jak-STAT pathway has not been cleared out yet. Hedges and Johnson (2008) did demonstrate that double stranded RNA, nor inactive DCV particles induced a Jak-STAT response. Virus replication and its non-structural proteins are likely candidates for virus recognition. Moreover, the mechanisms that Jak-STAT ultimately employs to clear the infection have not yet been identified either, but the effect is a general, virus non-specific response (Hedges & Johnson 2008). A good option would be apoptosis. As mentioned, the Jak-STAT original function lies in the embryonic development. Apoptosis is often an essential process in the development of organs and limbs, but is moreover an effective antiviral response.

Apoptosis is one of few mechanism of insects to control virus infections that has been demonstrated is apoptosis (Clem *et al.* 1991). Apoptosis is an energy dependent process of cell suicide characterized by a number of distinct morphological features and biochemical processes (e.g. cell shrinkage, plasma membrane blebbing, intra-nucleosomal cleavage) that will ultimately lead to cell fragmentation into apoptotic bodies (O'Brien 1998). Developmental cues or a damaged metabolism are main triggers for programmed cell death, but viruses can cause apoptosis as well.

In mammals, apoptosis of virus-infected cells is either induced by T-lymphocytes that recognize viral peptides or apoptosis is induced autonomously when infected cells detect unscheduled cell cycle activation (O'Brien 1998). T-lymphocytes are unavailable to the innate immune system of insects and activity of hemocytes, capable of recognizing non-self tissue, has

not been correlated to virus intrusion. The autonomous mammalian mechanism has not been found in insects either.

A more likely candidate-mechanism for virus-induced apoptosis was identified this year (Settles & Friesen 2008). When the *Drosophila* flock house virus replicates, it reduces normal protein synthesis in infected *Drosophila* cells. This causes the unstable and naturally short-lived *Drosophila* inhibitor-of-apoptosis protein (DIAP1) to drop significantly in concentration level, thereby stopping inhibition of the apoptotic process and triggering the cell's death. Thus will the virus not be able to propagate effectively. The study also indicates that this mechanism is not species-specific and can be used against a broad range of viruses (Settles & Friesen 2008). This sounds intuitive; any virus that replicates needs the cellular machinery for copying. Unless the virus can provide other resources the cell's production of its own proteins will go down causing DIAP1 to drop. This would be a highly virus species independent mechanism, as most (harmful) viruses replicate.

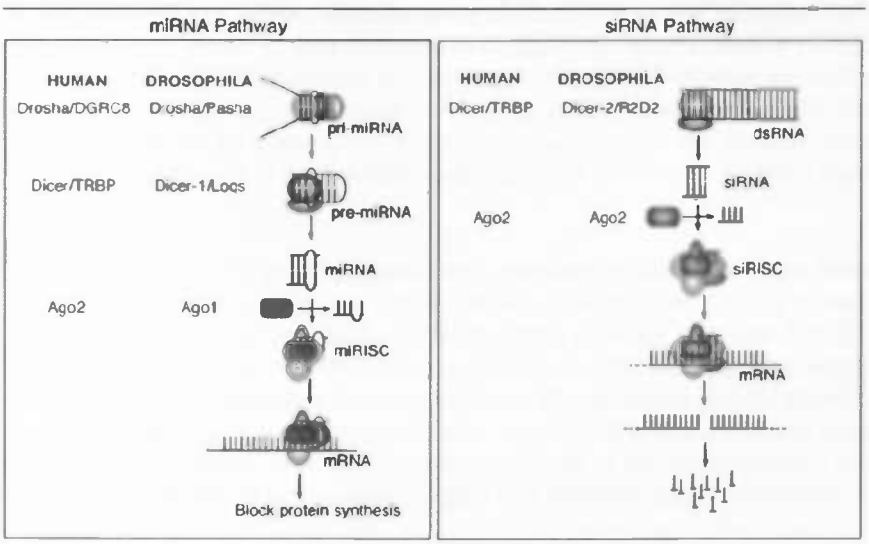

Figure 2. The two RNA silencing pathways as used by Drosophila (in red) and the human orthologs (in blue). Where the miRNA pathway only blocks further translation of the RNA, siRNA effectively destroys the foreign RNA. From Marques & Carthew (2007)

Many processes in the eukaryotic cell are regulated by RNA silencing. Micro RNA (miRNA) and small interfering RNA (siRNA) both inhibit the translation of messenger RNA by means of antisense binding. Where miRNA merely binds to mRNA thus blocking translation, siRNA is incorporated into a RNA induced silencing complex (RISC) which then binds to the targeted mRNA and ultimately cleaves it (Huttenhofer *et al.* 2005). RNA interference (RNAi) as an important process in regulating the cell's metabolism is in itself still a recent insight (Dennis 2002). Yet, new found evidence leads scientists to believe that the original function of RNAi was in fact inhibiting viral replication (Cherry & Silverman 2006).

RNA interference as an antiviral response has been found plants and *Caenorhabditis elegans* (Cherry & Silverman 2006). As all eukaryotes, insects utilize RNAi, have the required machinery and in 2006 two papers demonstrated that in *Drosophila melanogaster* RNAi is indeed used to counter viral infections (Zambon *et al.* 2006,Wang *et al.* 2006). Both papers showed that *Drosophila*-lines with knock-outs in the RNAi pathway had an increased virus titer level and a decreased survival, thereby linking virus susceptibility with the process of RNA silencing.

The two papers share their main conclusion, yet they differ in details. Where as Wang *et al.* (2006) state that Dicer2 is essential for cleaving the viral RNA, Zambon *et al.* (2006) state that the function of Dicer2 is redundant with Dicer1, which carries more importance in the process. Moreover, Zambon *et al.* find that RNAi is not sufficient to clear the infection and that in fact the

Toll-pathway is activated to eventually get rid of the infection. The Toll-pathway has not been associated with the antiviral response before and Wang et al. do not mention it in their study. An explanation for this might be that the two papers used different viruses (Zambon *et al.* *Drosophila* X virus, Wang *et al.* flock house virus) that induce different reactions. Mere different strains of the Sindbis virus already induce different transcription factors in the mosquito *Aedes aegypti* (Campbell *et al.* 2008). But RNA silencing is supposed to be a virus unspecific response and indeed Wang *et al.* (2006) find results for the cricket paralysis virus to be identical to the results of flock house virus.

Even though the RNAi reaction might not be identical across viruses, there is a consensus on the general mechanisms at work. As a virus enters a cell, it releases its genome. Independent of the viral genome's nucleic acid (retroviruses excluded) a by product of viral replication is double stranded RNA (dsRNA), which is released in the cytoplasm (Marques & Carthew 2007). Triggered by their presence the RNaseIII enzymes (Dicer1 or Dicer2) cut the viral dsRNA into small sequences of 21-28 bp. siRNA. Subsequently r2d2 facilitates the incorporation of such a siRNA into the argonaut2 which is part of the RNA induced silencing complex (RISC). This complex uses the siRNA antisense to find and destroy other viral RNA thus preventing assembly of new viruses (Cherry & Silverman 2006,Zambon *et al.* 2006,Wang *et al.* 2006). Studies on *Anopholes gambiae* and *Aedes aegypti* and their respective viruses the Sindbis virus and the O'nyong-nyong virus confirm this mechanism in mosquitoes (Keene *et al.* 2004,Campbell *et al.* 2008).

The Jak-STAT pathway, cellular apoptosis and RNA interference the only three processes known to be involved in viral immunity. It is not always known how they are exactly activated, but they seem all rather general viral species unspecific responses. RNAi for example functions against single and double stranded RNA and DNA viruses. A species specific response would not necessarily have to mean an acquired immune system; the response can be genetically fixed. But if an insect wants to keep up with this evolutionary race with the fast mutating virus, it needs to target conservative areas in the virus. These generally tend to be characteristics shared by even distantly related virus families. Thus resulting again in a general, albeit apparently effective, response.

Resistance against viruses can also be acquired by physiological or behavioural means. Lepidopteran larvae have been observed to slough off midgut cells to get rid of an infection with a baculovirus (Cory & Myers 2003). In the same butterfly-virus system the hormonal homeostasis appears to influence the level of larvae resistance (Cory & Myers 2003). On the other hand there is no evidence suggesting that these larvae modify their behaviour to reduce ingestion of the baculovirus (Cory & Myers 2003).

Viral evasion of the immune system and resulting arms race

The only evolutionary successful virus is a persistent virus. One way of increasing ones chance for success is to replicate. To achieve this, a virus has to take the cell's machinery hostage. This has detrimental effects on the host, so it will try to prevent the virus from reproducing successfully. Mechanisms applied by insects to prevent virus dispersal have just been discussed, but of course viruses have means of their own to evade the insect immune system. This has the potential of setting off an arms race between the host's immune response and the ability of viruses to circumvent these obstacles.

Apoptosis is a natural process and every eukaryotic cell has the potential to induce it. To prevent accidental activation of this cascade, cells produce suppressors of apoptosis (also called tumour suppressor proteins). Clem *et al.* (1991) demonstrated that a baculovirus infecting its Lepidopteran host *Autographa californica* can inhibit apoptosis as well. It encodes the p35 protein that, once translated, forms a stable complex with a broad range of caspases, inhibiting further progress of cell death and securing a successful replication cycle (O'Brien 1998). Evidently this brings the selection pressure back on the insect host. In the fruit fly the first counter measure has been observed; a *Drosophila* caspase (DRONC) has developed resistance against p35 (Clem 2001).

Baculoviruses employ several other apoptosis inhibitors but not all trigger such evident arms races. More than ten species of baculovirus carry a gene from the family of Inhibitors of Apoptosis (IAP) which has, as its name predicts, an anti-apoptotic function (Clem 2001). These genes are homologous to the IAP genes of their Lepidopteran hosts which led to the hypothesis that once or several times in their evolutionary history the virus had captured these IAP from its host. In a phylogenetic study Hughes (2002) compared the phylogeny of a range of baculoviruses with their IAP and their hosts' homologues. It showed that at least two independent gene-capturing events have occurred. One expects that this sets off an evolutionary arms race where the host IAP evolves away from the pathogen's counterpart. However, no difference in amino acid replacement rate was found between the pathogen and host IAP.

Moreover, of all the immune related genes, IAP had the lowest evolutionary rate. These captured IAP homologues are therefore unlikely to have driven an evolutionary arms race (Hughes 2002). Of course, mutating IAP also requires a mutation in the next step of the cascade, so it can still recognize IAP. The virus on the other hand does not have this constrained and moreover mutates much faster, making this a very unfair race. This could explain why the arms never got of the ground and thus no traces of a high evolutionary rate.

RNA interference is also subject to viral suppression. The flock house virus (FHV) carries a gene for b2, a suppressor of RNA silencing (SRS). b2 binds to both dsRNA and siRNA, thus inhibiting dicer2 activity. This makes b2 essential for FHV replication (Li *et al.* 2002). A b2 knock-out FHV-strain can only cause infections in dicer2 knock-out *Drosophila,* though these infections are much less severe. This suggests that the b2 protein serves multiple purposes for the virus (Marques & Carthew 2007). Many other viruses use SRS and the majority of these SRS target early steps in the RNAi pathway like dicer2 or r2d2 (Marques & Carthew 2007). Obbard *et al.* (2006) showed that dicer2, r2d2 and ago2 evolve much faster than their paralogues involved in 'housekeeping' miRNA silencing. In fact, the three genes belong to the fastest evolving 3% of the *Drosophila* genome. Moreover, the number of polymorphic sites was extremely low, indicating strong positive selection on these genes (Obbard *et al.* 2006).

Coevolution

The family of Baculoviridae knows two genera, the nucleopolyhedraviruses (NPV) and granuloviruses (GV), that can infect a broad range of insects. Infection occurs horizontally by ingestion of occlusion bodies filled with virus particles that can be encountered around previous victims' cadavers (Cory & Myers 2003). Did this virus infect new insect species by such transmission, or has the baculovirus accompanied the insects from the earliest ancestor and speciated with it? A phylogenetic comparison of the baculoviruses and their insect hosts shows that the GV phylogeny concords with the phylogeny Lepidoptera. The NPV phylogeny resembles the phylogeny of Diptera and Hymenoptera where the NPV of Diptera (closer relatives of Lepidoptera) are also more closely related to GV. The baculovirus and insects therefore must have started their relationship in early evolutionary history and coevolved from there (Herniou *et al.* 2004).

Coevolution is the process of reciprocal, adaptive genetic change between interacting species (Woolhouse *et al.* 2002). Traits are susceptible to coevolution when both the pathogen and host traits contain genetic diversity, as with 'normal' evolution. But furthermore, the traits need to have reciprocal effects on the fitness of the two interacting species, and the outcome of these interactions needs to differ per combination of interacting genotypes (Woolhouse *et al.* 2002). The pathogenic and resistance factors of virus and insect-host both definitely show genetic variation. A virus has by definition a negative effect on host fitness and depending on the level of resistance, the host determines the fate of a virus infection. Viruses and their insects (as with any pathogen and host) are subject to coevolution.

In the battle between *Drosophila* and FHV we see this process of reciprocal adaptive genetic change. FHV infects and kills *Drosophila* cells, the cells in response develop RNAi, blocking virus reproduction. The virus in his turn counters this again using an inhibitor of RNAi. The two species are clearly coevolving. Yet how this looks on a population level remains unclear. The process of coevolution is hard to observe on the level of a population; evidence consists merely of patterns of genotypic variation that are in concordance with coevolution (Woolhouse *et al.* 2002).

Different evolutionary mechanisms predict different genetic patterns of coevolution. When there is a cost to virulence and resistance, models predict negative frequency dependent selection where rare polymorphisms have an selective advantage in the population (Antonovics & Thrall 1994,Simms 1996). Costly resistance has been proven in molecular studies (e.g. maintaining the RNAi machinery; Woolhouse *et al.* 2002), but cost-free resistance has been documented in several cases as well (e.g. a mere mutation at the recognition site of a receptor; Simms 1996). When there is strong directional selection, one allele will get fixed in the population through a selective sweep depleting genetic diversity (Summers *et al.* 2003). For example, the low level of polymorphism found around the genes of the *Drosophila* RNAi machinery suggests that a selective sweep passed through that population (Obbard *et al.* 2006).

How fast are coevolutionary dynamics? Bangham *et al.* (2007) studied the dynamics of sigma virus and *Drosophila melanogaster*. The virus, which can only be transmitted vertically from parent to offspring, reduces the viability of eggs and increases sensitivity to carbon dioxide. A single albeit complex mutation in the gene *ref(2)P* causes resistance in *Drosophila* flies. Bangham *et al.* (2007) found that positive selection has acted on *ref(2)P* since it mutated 1000-7000 years ago.

However, since the 1980s a dramatic increase was witnessed of a resistant genotype of sigma virus, unaffected by the *ref(2)P* mutation. The lag between host-resistance and virus-counter adaptation is in this case interesting. One explanation the authors propose is that selection on the virus for counter-adaptations has been very weak; the *ref(2)P* mutant has a very low gene-frequency and an individual needs to be homozygous to be resistant. Thus sigma virus hardly encountered resistance until the 1980's (Bangham *et al.* 2007). It is interesting to keep following this process; will the *ref(2)P* frequency increase ever faster in the population until it is

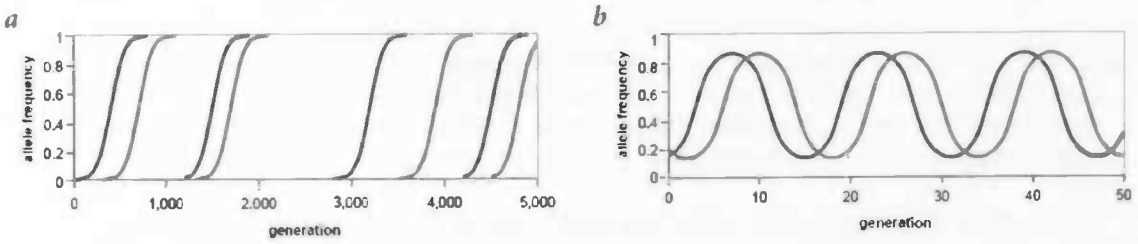

Figure 3. Allele frequency changes driven by coevolution. ***a*** *A series of selective sweeps where the blue and red line can signify either the virus or the host. As new successful allele (an new way to evade the immune system or a new immune response) is introduced to the population, it quickly spreads (blue). To this the other population responses with a counter-adaptation. Polymorphisms can be found at any stage in one, both or non of the populations.* ***b*** *Frequency dependent selection. Again both virus or host can be the blue or red line. Here first the adaptive allele spreads through the population (blue) but as it become to prevalent, the counter adaptation gets an selective advantage and will thus be selected for (red). As this allele increase, the selective advantage for the first allele diminishes and its frequency drops again, etc. etc. In this scenario at all times polymorphisms will be found in both populations. From Woolhouse* et al. *(2002)*

fixated, followed by the resistance of sigma virus? Or will the *ref(2)P* frequency diminish again before fixation but remain in the population at a low level, because the sigma virus resistance spread.

Evolution is a dynamic process, which makes recognition of modes of coevolution more challenging. What we observe in nature today is not the end product of coevolution but can very well be a population currently undergoing a selective sweep. The observed polymorphisms would then not be the product of frequency dependent selection but genotypes that have not yet been weeded out of the population (Woolhouse *et al.* 2002).

Several other factors can severely complicate the matter of demonstrating coevolutionary processes in wild populations. The genetic basis of the virus-insect interaction might not be as straightforward as in the *ref(2)P* example where one gene for resistance complemented the corresponding gene for virulence. Many resistance mechanisms battle more than one pathogen species (e.g. RNAi) and therefore cannot easily adapt to one particular virus without any consequences for all others. Furthermore, interactions are not one-on-one; multiple viruses (co)infect multiple insect species. Trade-offs between resistance/virulence traits and competition among strains prevent the coevolution of two species (Woolhouse *et al.* 2002,Little 2002). Co-infections can furthermore cause recombination and exchange of genetic material (Cory & Myers 2003). Finally, large environmental variance (e.g. different encounter rates, maternal effects) and phenotypic plasticity can cloud the whole effect of genetic evolution (Little 2002). An adaptive immune system is a good example of phenotypic plasticity. This inhibits any further evolutionary adaptation to the pathogen until the immune response is not adequate any more. Since the adaptive does seem adequate for vertebrates, one would expect to see much less traces of coevolution in genes of the vertebrate immune system and their viruses.

Life-history

A virus never comes alone. Even if it does start off alone, any effective virus will within no time be with billions. And it is with this excess of viruses that an insect will start noticing the effect. These will mostly be negative fitness effects, changing the individual's future life history. The exact consequences of an infection differ, as with any pathogen, from virus to virus ánd depend on the host species.

18 species of virus are known to infect bees and while some have exotic names like Kashmir bee virus or bee virus X, other names give a general idea of potential virus symptoms: acute bee paralysis virus, chronic bee paralysis virus, black queen cell virus, sacbrood virus, filamentous bee virus, deformed wing virus and cloudy wing virus (Chen *et al.* 2006). Despite the morphological variations these viruses induce, all viruses increase bee mortality rates (Evans & Hung 2000).

Generally, insect viruses decrease the host fitness through either an increased mortality (e.g. *Drosophila* X virus; Zambon *et al.* 2006), a decreased fecundity (e.g. sigma virus; Bangham *et al.* 2007) or both (e.g. granulovirus; Cory & Myers 2003). Many viruses cause a range of sublethal effects like slower development and reduced maturation size (Kukan 1999). But there is a notable exception. Fruit flies infected with *Drosophila* C virus c-strain do not only shorten their developmental time but also increase their fecundity. The virus can be considered a symbiont, but only in the adult stage; the DCV causes a high rate of juvenile mortality. The virus does not select for fast growing, fertile fruit flies. It really is an effect of the virus. (Gomariz-Zilber & Thomas-Orillard 1993).

Why are so many viruses detrimental to their host? Is it not better to increase the number of potential hosts instead of reducing them? Especially a vertically transmitted virus like sigma virus should increase its host fecundity instead of reducing the viability of the eggs. A replicating virus needs a cell to do the work. This costs the cell resources. So there will always be some cost to replication. A virus is perhaps able to target particular cells or life stages of the host to minimize its damaging impact. Ineffectively transmitted viruses could be more 'gentle' to their host thus increasing the host's offspring which increases the future encounter rate and chance of transmission.

Viruses influence insect life-history, but the reverse is also true. Insects can direct virus life-history. 'Life-history' might be an awkward term for a single, lifeless virus particle, but the term suits quite well if applied to the entire infection; it has to grow, use resources and in the end reproduce. For an infection, reproduction means to successfully transmit itself to a new host. For this it has one of two options, or even (Kukan 1999) a combination of the two. Vertical transmission occurs exclusively from parent to offspring. Virus particles are transmitted through the gametes ending up in the eggs or in many cases the particles are deposited on the eggs' surface (Kukan 1999,Chen *et al.* 2006). Horizontal transmission occurs between any two individuals and is facilitated by aerosols, ingestion or vectors carrying the virus (Chen *et al.* 2006).

As previously stated, in vertical transmission the mother or both parents can infect offspring. But this transmission is not a mere chance process for the virus; the parent insects can have a big impact on its success. The sigma virus can be transmitted by both the mother and the father *Drosophila melanogaster*. In females the transmission success correlates with the presence of the *ref(2)P* resistance gene and thus likely only depends on viral load. But the chance for a virus to be paternally transmitted depends not on the males viral resistance through *ref(2)P*, but on a variety of other unknown genetic components (Bangham *et al.* 2008). For a sigma virus infecting male flies a high within host replication rate (mediated by *ref(2)P*) is therefore apparently not important for its continuity and the virus should instead focus its transcription on successful transmission to the sperm, which ís essential.

Interestingly Kukan (1999) includes transstadial transmission in vertical transmission, despite its definition. What is moreover interesting is that this type of transmission is not a given

for viruses; many infected larvae leave the pupal stage uninfected. Metamorphosis might interrupt the virus replication cycle, perhaps by hormonal responses (Kukan 1999). Such a clearance of infection would only be available to organisms that undergo a transitional state.

The opposite was also found; not infected larvae matured in infected adults. If the insects had not accidentally been contaminated during the experiment, the most likely explanation is that the virus infection has been in a latent, or low replicating, state (Kukan 1999). The study by Anderson and Gibbs (1988), mentioned in the introduction of this paper, demonstrates the presence of viruses in honey bees that do not show any sign of infection. The mechanism is not exactly known, but several stress factors are suspected to activate the virus to become virulent again (Chen *et al.* 2006), though others claim it is a stochastic process (Cory & Myers 2003).

The known viral detection mechanisms in insects can only detect 'active' viruses; those that are replicating (for an example: Hedges & Johnson 2008). A latent virus should therefore go unnoticed, which it evidently does often. In contrast, in vertebrates T cells can recognize viral receptors on infected cells, independent on whether the virus is active. A difference in the number of latent viruses encountered in vertebrates or insects might be caused by this difference of virus detection mechanism. One other difference with human viruses that can go in a latent state like human immunodeficiency virus or herpes is that insect viruses can remain latent over several generations.

Horizontally transmitted viruses tend to be more pathogenic (Chen *et al.* 2006). A vertically transmitted virus has to keep its insect host alive until it has reproduced. For horizontal transmission the tradeoff between replication within and between hosts is much more in favour of within-host replication. But there is an optimum; when the infection is too virulent, between-hosts transmission will drop again due to death of the virus carrier before any transmission opportunities arose. However, if an insect would get infected with several virus strains, models predict a competition among strains. High replicating mutants will dominate less virulent strains which leads to an overall more severe infection for the insect. The infection will die out though, since its between hosts transmission is hampered (Bonhoeffer & Nowak 1994). This could explain the solitary infection observed in all bees of the Anderson and Gibbs (1988) experiment, though the winner of the internal competition must have differed per host.

Insects generally harbour several strains of the same virus. In vivo cloning revealed 24 genotypically distinct strains of NPV within a single beauty moth *Panolis flammea* (Cory & Myers 2003). The resulting competition does not only lead to the constant within host evolution of more virulent infections, the infection can even reproduce in the moth population. This is possible because NPV produces occlusion bodies for dispersal to other hosts. Within such an occlusion body, several genetically different viral genomes can be present if they infected the same cell. This gives rise to parasitic viral genomes that concentrate their activity on genome replication causing higher virulence (Bull *et al.* 2003). The *Lepidopteran* victim can do little to prevent this; with the ingestion of one occlusion body several viral strains enter the body.

Insects do have other ways of influencing the transmission rate of viruses. Gregariously feeding larvae of the African armyworm *Spodoptera exempta* have a decreased transmission rate of NPV compared to their solitary living counterparts (Cory & Myers 2003). What type of plant the larvae feed on may also affect the efficiency of viral transmission and larvae are known to change diet in response of an infection (Cory & Myers 2003).

The ecological role of viruses and their insects

If a virus has repercussion for the life-history of a single insect, than what impact does a viral epidemic have on an entire population? There is huge amount of models generating all sorts of predictions on pathogen induced host population dynamics and even virus induced population cycles in insects (for an overview: Bonsall 2004). Thus several key factors have been identified that should significantly alter the insect population dynamics as determined by viral frequencies; high virus induced mortality, large virus yields, relatively small rates of population growth and a long persistence time of the virus latent state.

Lab experiments have tried to recreate the predicted virus induced cycles in insect populations. Sait *et al.* (1994) for example registered for almost two years the demographic properties of six small lab-reared populations *Plodia interpunctella.* At the start of the experiment, half of the populations were exposed to the remains of granulovirus killed larvae. What was immediately evident was that population cycles do not require the presence of a virus. The virus does make the cycles longer and more stable (Sait *et al.* 1994). A more in-depth analysis of similar time series revealed that the competition and cannibalism between large and small larvae is responsible for negative density dependence, creating the cycles. The dominant effect of the granulovirus on these dynamics is not so much its induced mortality as its sublethal effects (inhibition of growth and its reduction of reproduction) stabilizing the population cycles (Bjornstad *et al.* 1998). The mechanism of density dependent feedback is however context dependent. Where in the singly infected population the growth of larvae shows density dependence, an added virus does not only increase the speed of kill, but makes death rate linearly dependent on larvae density (Bonsall & Benmayor 2005).

So insect viruses are not the main cause of population cycling, but what is their role in ecology? There is no doubt that insects fulfill a major role in ecosystems, even if it was only for their abundance. A global phenomenon is the population cycle shown by forest *Lepidoptera* where the population size can change up to a 10,000-fold and cause major defoliation. This is not a species specific trait but dependent on the ecology since the species' dynamics change with geographical gradients. External forces like climate differences are not the cause of this; the effect looks caused by delayed negative feedback loops (Berryman 1995). As shown in the previous lab experiments, viruses are not likely candidates for this. Field observations confirm that, although in some years viral epizootic fluctuations follow the population dynamics, these epizootics can be absent without interrupting the cycle (Berryman 1995). Liebhold *et al.* (2000) on the other hand argue that at high *P. interpunctella* density viruses are the main controlling agent, causing collapse of the population. At lower densities small mammalian predators take over the mediating role. Viruses may therefore not control the whole cycle but are vital for its completion (Liebhold *et al.* 2000).

It is interesting to see how the field and lab results contradict all these model predictions. Still there is a lot of theory produced that predicts pathogen induced population cycles. Is the forest *Lepidoptera* system the odd one out? Are mechanisms for insects and their viruses fundamentally different? Perhaps the difference in immunity mechanisms can be responsible for different dynamics on this much larger scale. Or perhaps theory just has trouble with keeping up with the field.

Most of the paper has dealt with viruses killing insects. But during evolutionary history insects and viruses have also found each other in unlikely partnerships. The insect as a vector or the virus as a symbiont have become important factors in ecology.

Insects do not only have to deal with their own viruses, they are one of the most important taxons of virus vectors, carrying viruses from one host to another. Both plant and animal viruses use them as intermediates. Mosquitoes are for humans probably the most notorious vectors, carrying dengue, yellow fever and Japanese encephalitis. Vertebrate viruses that are carried by a blood sucking arthropod and that can replicate in both arthropod and vertebrate are known as arthropod-borne viruses (arbovirus; Kuno & Chang 2005).

Aedes aegypti is vector of, among plenty other arboviruses, the Sindbis virus. The virus causes pains in the joints and rash in humans when infected. *A. aegypti* activates the RNAi response but cannot prevent the virus from replicating in the salivary gland. For the virus this is important because it infects new human hosts through the saliva an mosquito injects before drinking. The RNAi does not decrease mosquito mortality either (Campbell *et al.* 2008). However, if the virus is hardly virulent in the vector (as to secure its passage to a new host), mortality is not expected to have increased either. Sindbis virus can thus safely be transmitted from human to human.

But arboviruses do have negative effects on their insect vector. Eastern equine encephalomyelitis (EEE) is transmitted between birds or mammals by the mosquito *Culiseta melanura.* When EEE enters the mosquito it starts to replicate massively. This reduces the mosquitoes fecundity and survival dramatically, but only after two weeks; *C. melanura* has the highest probability of refeeding (and transmitting EEE) within 10 days, thus the virus propagation is hardly reduced. The reason for replication within the mosquito was not found (Scott & Lorenz 1998). If such a severe replication is not necessary for reaching the salivary gland, it might just be a spill-over effect of adaptations needed in the vertebrate host.

Almost all plant viruses transmitted by insects do not have detrimental effects on their vector. They also do not propagate in their insect vector. The three plant virus families that do replicate within insects, and have a negative fitness effect, are closely related to arboviruses (Gray & Banerjee 1999). That a virus does not replicate in the vector might actually be adaptive and not only the result of a tradeoff between plant and animal specialization. Not replicating, inactive viruses will go unnoticed by the insect's immune system (Hedges & Johnson 2008) and therefore increase their change of reaching a new plant host.

Plant viruses can be transmitted from the vector in a multitude of ways, yet are much less efficient in transmission than their vertebrate analogs. This is because they cannot pass the cell membrane independently and need the insect to damage the cell, but without killing it. The viruses are released either through the salivary gland which they entered via the gut, or they are released from the stylet of foregut. Even though these latter viruses are residing around the mouth, their transmission is not merely mechanical but involves molecular factors ensuring a successful persistence in the insect's mouthparts (Gray & Banerjee 1999).

One type of insect that has not been mentioned yet in this paper is a parasitiod itself: the parasitic wasp. Generally a female wasp catches specific host species larvae that she paralysis to subsequently lay her eggs in. These eggs develop in larvae themselves that feed on the host larva they reside in, killing it from the inside. The problem for these parasitoids is that a larva has a functional immune response that can encapsulate the parasitoid's eggs. The ovipositing female wasp can avoid the host immune system by laying her eggs in places where they are safe from any immune reaction. One good example is laying eggs outside of the host. Of course the host immune system will still get activated when the larvae start feeding, but encapsulation of the parasitoids is impossible. Another safe place to lay eggs is the nerve ganglion, where the host's hemocytes can not come. The female wasp can also coat her eggs with the same basement membrane factors that the host has around its tissues, thereby making them unrecognizable for the hemocytes (Schmidt *et al.* 2001).

Many hymenoptera endoparasitiods have a more active approach to evade the immune system. When ovipositing, these females inject polydnaviruses together with their eggs. Once these virus particles have entered host cells, they can suppress encapsulation of the parasitoids eggs. The virus particles do not replicate in the host but keep transcribing encapsulation inhibiting factors until the larvae have fully developed. The virus is strictly vertically transmitted and seems only to reproduce in the female wasp ovary (Schmidt *et al.* 2001). The symbiosis between wasp and virus is so tight that part of the viral genome resides in the wasp's genome. Moreover, the viral genome contains over 70% non-coding DNA and introns, both of which are unusual in viruses (Espagne *et al.* 2004). Within the ichnovirus (one of the two genera of polydnavirus) a strong diversifying selection has been found on sites that are associated with

host immunity. Especially binding sites show a high divergence. These sites are most likely involved in the arms race with the Lepidopteran larvae host immune system (Dupas *et al.* 2003). Interestingly the unlikely symbiosis between wasp and virus is by some suspected to be the main mediator of the delayed density dependence in forest populations of *Lepidoptera* (Berryman 1995).

Synthesis

This paper has tried to give a general but extensive idea what can happen when a virus is confronted with an insect. The insect's antiviral molecular pathways have only started to be identified. The recognition of viruses is not always clear as are the ultimate processes of destroying the virus. If viruses are not destroyed, they will multiply. Yet for many viruses this internal spread of the infection causes symptoms in the insect's phenotype characteristic to that virus. This pathological pattern might have to do with the specific cells a virus is targeting. General effects of an infection are reduced insect survival and fecundity. Though this is not always the case, which raises the question why it does seem to be a logic consequence of infection. What exactly causes the reduction in fecundity?

Mortality and reproductive rate are two important population parameters. The exact pathology has therefore theoretically a large impact on the dynamics of an epidemic. However, the prevalence and fluctuation of an epidemic are not the main determinants of the host population dynamics. Other ecological factors and especially the population's own density dependence all influence the population fluctuations. Virus prevalence presumably only plays a modest role in this. Yet all these ecological factors are interconnected and the complicated interplay has not been untwined. Especially being vectors of other diseases makes the insect-virus ecological role not only a major role, but an inherently dynamic process.

While more and more details of virus-insect interactions are being identified, especially between fields large gaps remain. A full integration from the molecular to ecological level of virus insect dynamics can not be realized yet. For example, where molecular studies demonstrate a clear relation between the diversity of parasitism and genetic variation in the host, ecological studies have problems finding this pattern in the outside world (Summers *et al.* 2003).

Another problem is the question of generality; the studies used for this overview use a variety of insect (and virus) species. Most molecular studies are done on *Drosophila melanogaster*. Yet the more pathological studies are on bees, probably because of their economic value and long relation with human society. Can ecological processes concerning the gypsy moth ever be explained using genetic data of fruit flies? The insect immune response is most likely quite conservative and can be assumed to generally function the same throughout the insect class. But at higher levels, things get less and less general. Especially ecological dynamics will be hard to generalize, because responses are will be less defined by the basic virus-insect interaction. The insect species phenotype will start playing a much bigger role. And insects are a very diverse group. Studying a broad range of insect species is therefore important to filter out the core effects of insect viruses in ecology.

Insects and their viruses are an ideal model system to study disease dynamics. Insects have relative short generation times, have much offspring and are small. This makes it is easy to maintain large populations in the lab. Compared to any mammal-disease model the chance of cross infection of a virus from insect to human is low, although there are notable dangerous exceptions (e.g. Sindbis virus).

Lab results can without any effort be exported to the field and tested on abundant wild populations if a common species is used. Mathematical model assumptions like the "mass action" rule have already been tested using insect populations (D'Amico *et al.* 1996). Moreover, creating specific disease models for insects is relatively straightforward, because of their lack of acquired immune system (Anderson & May 1980).

The molecular arms race between virus and insect leads to a coevolutionary process. Virus-insect dynamics allows us to study this process in great detail. Its great advantage over any vertebrate-disease system is that with insects the acquired immune system cannot inhibit coevolution or cloud any results. And because of fast insect reproduction and high virus mutation mutation, it is 'coevolution while you wait' (Oldroyd 1999).

Viruses have been suggested as pest control. Effective as some epidemics might be at eradicating local populations, a word of caution is at place. Lab and field studies have indicated that viruses stabilize the population cycling effect, but also exacerbate it (Sait *et al.* 1994,Berryman 1995). Insect-caused crop damage would therefore only become bigger in peak years under influence of an infection.

A virus might not be so efficient to control insect pests, it might proof very useful in preventing viral epidemics. In the introduction it was mentioned that one virus in a honey bee can prevent the other present viruses from replicating (Anderson & Gibbs 1988). Many human diseases also replicate in the mosquito vector (Campbell *et al.* 2008). If an infection with a different (to humans harmless) virus could suppress this within-vector replication, viral epidemics like dengue could be severely reduced. This technique would not be applicable to plant viruses, since most of these viruses do not replicate in their insect vector.

A last application of the insect-virus model often mentioned is to use it as a template for human immunity and disease dynamics. It allows for studying purely effects of the innate immune response and its role in the immune system. But the human and insect innate immunity are not the same; evolution did not stop for invertebrates after vertebrates developed T-lymphocytes. RNA interference in insects functions for example more efficiently by destroying viral RNA using siRNA, where in mammals the viral RNA translation is merely inhibited by imperfect fit of miRNA (Marques & Carthew 2007). How much this difference influences large scale dynamics is not known. Understanding the full consequences of using siRNA for silencing in virus-insect interactions would be a good start.

References

Anderson, D. L., and A. J. Gibbs. 1988. Inapparent virus-infections and their interactions in pupae of the honey bee (Apis mellifera Linnaeus) in Australia. Journal of General Virology **69**:1617-1625.

Anderson, R. M., and R. M. May. 1980. Infectious-diseases and population-cycles of forest insects. Science **210**:658-661.

Antonovics, J., and P. H. Thrall. 1994. Cost of resistance and the maintenance of genetic-polymorphism in host-pathogen systems. Proceedings of the Royal Society of London Series B-Biological Sciences **257**:105-110.

Bangham, J., K. W. Kim, C. L. Webster, and F. M. Jiggins. 2008. Genetic variation affecting host-parasite interactions: Different genes affect different aspects of sigma virus replication and transmission in Drosophila melanogaster. Genetics **178**:2191-2199.

Bangham, J., D. J. Obbard, K. W. Kim, P. R. Haddrill, and F. M. Jiggins. 2007. The age and evolution of an antiviral resistance mutation in Drosophila melanogaster. Proceedings of the Royal Society B-Biological Sciences **274**:2027-2034.

Berryman, A. A. 1995. What causes population cycles of forest lepidoptera? Trends in Ecology and Evolution **11**:28-32.

Bjornstad, O. N., M. Begon, N. C. Stenseth, W. Falck, S. M. Sait, and D. J. Thompson. 1998. Population dynamics of the Indian meal moth: Demographic stochasticity and delayed regulatory mechanisms. Journal of Animal Ecology **67**:110-126.

Bonhoeffer, S., and M. A. Nowak. 1994. Mutation and the Evolution of Virulence. Proceedings of the Royal Society of London Series B-Biological Sciences **258**:133-140.

Bonsall, M. B. 2004. The impact of diseases and pathogens on insect population dynamics. Physiological Entomology **29**:223-236.

Bonsall, M. B., and R. Benmayor. 2005. Multiple infections alter density dependence in host-pathogen interactions. Journal of Animal Ecology **74**:937-945.

Bull, J. C., H. C. J. Godfray, and D. R. O'Reilly. 2003. A few-polyhedra mutant and wild-type nucleopolyhedrovirus remain as a stable polymorphism during serial coinfection in Trichoplusia ni. Applied and Environmental Microbiology **69**:2052-2057.

Campbell, C. L., K. M. Keene, D. E. Brackney, K. E. Olson, C. D. Blair, J. Wilusz, and B. D. Foy. 2008. Aedes aegypti uses RNA interference in defense against Sindbis virus infection. Bmc Microbiology **8**.

Chen, Y. P., J. Evans, and M. Feldlaufer. 2006. Horizontal and vertical transmission of viruses in the honeybee, Apis mellifera. Journal of Invertebrate Pathology **92**:152-159.

Cherry, S., and N. Silverman. 2006. Host-pathogen interactions in drosophila: new tricks from an old friend. Nature Immunology **7**:911-917.

Clem, R. J. 2001. Baculoviruses and apoptosis: the good, the bad, and the ugly. Cell Death and Differentiation **8**:137-143.

Clem, R. J., M. Fechheimer, and L. K. Miller. 1991. Prevention of Apoptosis by A Baculovirus Gene During Infection of Insect Cells. Science **254**:1388-1390.

Cory, J. S., and J. H. Myers. 2003. The ecology and evolution of insect baculoviruses. Annual Review of Ecology Evolution and Systematics **34**:239-272.

D'Amico, V., J. S. Elkinton, G. Dwyer, J. P. Burand, and J. P. Buonaccorsi. 1996. Virus transmission in gypsy moths is not a simple mass action process. Ecology **77**:201-206.

Dennis, C. 2002. The brave new world of RNA. Nature **418**:122-124.

Dostert, C., E. Jouanguy, P. Irving, L. Troxler, D. Galiana-Arnoux, C. Hetru, J. A. Hoffmann, and J. L. Imler. 2005. The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. Nature Immunology **6**:946-953.

Dupas, S., M. W. Turnbull, and B. A. Webb. 2003. Diversifying selection in a parasitoid's symbiotic virus among genes involved in inhibiting host immunity. Immunogenetics **55**:351-361.

Espagne, E., C. Dupuy, E. Huguet, L. Cattolico, B. Provost, N. Martins, M. Poirie, G. Periquet, and J. M. Drezen. 2004. Genome sequence of a polydnavirus: Insights into symbiotic virus evolution. Science **306**:286-289.

Evans, J. D., and A. C. Hung. 2000. Molecular phylogenetics and the classification of honey bee viruses. Archives of Virology **145**:2015-2026.

Gomariz-Zilber, E., and M. Thomas-Orillard. 1993. Drosophila-C virus and Drosophila hosts - A good association in various environments. Journal of Evolutionary Biology **6**:677-689.

Gray, S. M., and N. Banerjee. 1999. Mechanisms of arthropod transmission of plant and animal viruses. Microbiology and Molecular Biology Reviews **63**:128-148.

Hedges, L. M., and K. N. Johnson. 2008. Induction of host defence responses by Drosophila C virus. Journal of General Virology **89**:1497-1501.

Herniou, E. A., J. A. Olszewski, D. R. O'Reilly, and J. S. Cory. 2004. Ancient coevolution of baculoviruses and their insect hosts. Journal of Virology **78**:3244-3251.

Hoffmann, J. A. 1995. Innate Immunity of Insects. Current Opinion in Immunology **7**:4-10.

Hoffmann, J. A. 2003. The immune response of Drosophila. Nature **426**:33-38.

Hoffmann, J. A., J. M. Reichhart, and C. Hetru. 1996. Innate immunity in higher insects. Current Opinion in Immunology **8**:8-13.

Hughes, A. L. 2002. Evolution of inhibitors of apoptosis in baculoviruses and their Insect hosts. Infection Genetics and Evolution **2**:3-10.

Huttenhofer, A., P. Schattner, and N. Polacek. 2005. Non-coding RNAs: hope or hype? Trends in Genetics **21**:289-297.

Keene, K. M., B. D. Foy, I. Sanchez-Vargas, B. J. Beaty, C. D. Blair, and K. E. Olson. 2004. RNA interference acts as a natural antiviral response to O'nyong-nyong virus (Alphavirus; Togaviridae) infection of Anopheles gambiae. Proceedings of the National Academy of Sciences of the United States of America **101**:17240-17245.

Kukan, B. 1999. Vertical transmission of nucleopolyhedrovirus in insects. Journal of Invertebrate Pathology **74**:103-111.

Kuno, G., and G. J. J. Chang. 2005. Biological transmission of arboviruses: Reexamination of and new insights into components, mechanisms, and unique traits as well as their evolutionary trends. Clinical Microbiology Reviews **18**:608-637.

Lemaitre, B., and J. Hoffmann. 2007. The host defense of Drosophila melanogaster. Annual Review of Immunology **25**:697-743.

Li, H. W., W. X. Li, and S. W. Ding. 2002. Induction and suppression of RNA silencing by an animal virus. Science **296**:1319-1321.

Liebhold, A., J. Elkinton, D. Williams, and R. M. Muzika. 2000. What causes outbreaks of the gypsy moth in North America? Population Ecology **42**:257-266.

Little, T. J. 2002. The evolutionary significance of parasitism: do parasite-driven genetic dynamics occur ex silico? Journal of Evolutionary Biology **15**:1-9.

Marques, J. T., and R. W. Carthew. 2007. A call to arms: coevolution of animal viruses and host innate immune responses. Trends in Genetics **23**:359-364.

O'Brien, V. 1998. Viruses and apoptosis. Journal of General Virology **79**:1833-1845.

Obbard, D. J., F. M. Jiggins, D. L. Halligan, and T. J. Little. 2006. Natural selection drives extremely rapid evolution in antiviral RNAi genes. Current Biology **16**:580-585.

Oldroyd, B. P. 1999. Coevolution while you wait: Varroa jacobsoni, a new parasite of western honeybees. Trends in Ecology & Evolution **14**:312-315.

Sait, S. M., M. Begon, and D. J. Thompson. 1994. Long-Term Population-Dynamics of the Indian Meal Moth Plodia-Interpunctella and Its Granulosis-Virus. Journal of Animal Ecology **63**:861-870.

Schmidt, O., U. Theopold, and M. Strand. 2001. Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. Bioessays **23**:344-351.

Scott, T. W., and L. H. Lorenz. 1998. Reduction of Culiseta melanura fitness by eastern equine encephalomyelitis virus. American Journal of Tropical Medicine and Hygiene **59**:341-346.

Settles, E. W., and P. D. Friesen. 2008. Flock house virus induces apoptosis by depletion of Drosophila inhibitor-of-apoptosis protein DIAP1. Journal of Virology **82**:1378-1388.

Simms, E. L. 1996. The evolutionary genetics of plant pathogen systems - Understanding the coevolution of hosts and parasites is key to understanding their ecology. Bioscience **46**:136-145.

Summers, K., S. McKeon, J. Sellars, M. Keusenkothen, J. Morris, D. Gloeckner, C. Pressley, B. Price, and H. Snow. 2003. Parasitic exploitation as an engine of diversity. Biological Reviews **78**:639-675.

Tarpy, D. R., and T. D. Seeley. 2006. Lower disease infections in honeybee (Apis mellifera) colonies headed by polyandrous vs monandrous queens. Naturwissenschaften **93**:195-199.

Wang, X. H., R. Aliyari, W. X. Li, H. W. Li, K. Kim, R. Carthew, P. Atkinson, and S. W. Ding. 2006. RNA interference directs innate immunity against viruses in adult Drosophila. Science **312**:452-454.

Woolhouse, M. E. J., J. P. Webster, E. Domingo, B. Charlesworth, and B. R. Levin. 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature Genetics **32**:569-577.

Zambon, R. A., V. N. Vakharia, and L. P. Wu. 2006. RNAi is an antiviral immune response against a dsRNA virus in Drosophila melanogaster. Cellular Microbiology **8**:880-889.