Myrmecological News
ISSN 1997-3500
myrmecologicalnews.org
Myrmecol. News 30: 213-228 doi: 10.25849/myrmecol.news_030:213 21 October 2020
Review Article

Viruses and their effects in ants (Hymenoptera: Formicidae)

James W. Baty, Mariana Bulgarella, Jana Dobelmann, Antoine Felden & Philip J. Lester

Abstract

Viruses are ubiquitous within all forms of cellular life, including ants. We documented the currently known viral infections described and their effects on ants. Our literature review found 87 different viruses (including 40 putative viruses and five bacteriophages detected via high-throughput sequencing) across 38 ant species. The majority of these viruses have been described from studying pathogens as potential biological control agents for the invasive red imported fire ant, *Solenopsis invicta* Buren, 1972 or due to efforts to determine if ants serve as reservoirs for honey bee viruses in places where Argentine ants, *Linepithema humile* (Mayr, 1868) are also invasive. Most of these viruses belong to the Picornavirales order of small RNA single-stranded RNA (ssRNA) viruses, with more than half being positive-sense (+ssRNA) viruses. We review modes of viral transmission and suggest that horizontal transmission is a common mode of infection in ants as they share food via trophallaxis, although vertical transmission of viruses in eggs from queens has been observed. Viruses can substantially alter ant behaviour and physiology. We review effects of viruses on immune gene expression, feeding, locomotion, aggression, and colony defence. Then, we review the current state of the art in prospecting and using viruses for biological control. Mortality of ant colonies can occur, although the impact of some viral infections appears to be dependent on other environmental factors. Solenopsis invicta virus 3 (SINV-3) has had the most focus as a biological control agent. Effective laboratory and field transmission of SINV-3 in *S. invicta* colonies has been demonstrated although large-scale ant control with SINV-3 has not yet been reported. Finally, we review virus discovery and detection methods, including high-throughput sequencing that has revolutionised the field. We encourage testing for viral replication within each ant species to confirm active infection and that the ant is a true host to the virus, and we recommend approaches for viral discovery in invasive ants that focus on colony monitoring in their native range.

Key words: Viral pathogens, physiology, behaviour, population dynamics, viral discovery, review.

Received 11 June 2020; revision received 21 August 2020; accepted 7 September 2020
Subject Editor: Chris R. Smith

James W. Baty (contact author), Mariana Bulgarella, Jana Dobelmann, Antoine Felden & Philip J. Lester, School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand.
E-Mail: James.Baty@vuw.ac.nz

Introduction

Viruses are ubiquitous, parasitizing all cellular life forms, and are thought to represent the most physically-abundant and genetically-diverse biological entities on Earth (Koonin & Dolja 2013). Ants have been known to harbour viruses since the mid-20th century with reports of viral-like particles in the common wood ant *Formica lugubris* Zetterstedt, 1838 (Steiger & al. 1969), and in the fire ant, *Solenopsis* spp. (Avery & al. 1977). Since these publications, many different viral families have been discovered in different ant species, with much of the research typically being driven by the hunt for biological control strategies for globally important invasive ant species such as the red imported fire ant (*Solenopsis invicta* Buren, 1972) (Valles 2012, Oi & al. 2015). The objectives of this review are to: (1) summarise diversity, discovery, modes of transmission, and specificity of viruses infecting ants; (2) outline the effects of viruses on ant physiology and behaviour; and (3) discuss the potential of viruses for biological control of ants. Our goal with this resource is to encourage further research and highlight knowledge gaps ready to be explored.

The diversity, specificity, and phylogenetic relationships of viruses infecting ants

Our literature review of publications reporting viruses in ants showed 87 different viruses across 38 ant species (Tab. 1 and Tab. S1 as digital supplementary material to this article, at the journal's web pages). Many of the detected viruses have been found in the invasive Argentine ant *Linepithema humile* (Mayr, 1868) and red imported fire

Tab. 1: Summary of viruses reported to occur in ants. Viruses demonstrated to replicate in the ant host are shown in bold and with an asterisk. Viral replication demonstrates true parasitism of an ant host. Some of the viruses detected might not infect ants but were present following ingestion or for other reasons. In addition, 33 of the viruses listed were identified from partial sequences obtained via high-throughput sequencing. Viral detection methods and references are found in Table S1 as digital supplementary material to this article, at the journal's web pages.

Ant species	Viruses
Subfamily - Dolichoderinae	
Forelius sp.	Deformed wing virus (DWV)
Linepithema humile	Acute bee paralysis virus (ABPV), Alphabaculovirus, Aphid lethal paralysis virus, Black queen cell virus (BQCV), **DWV***, Drosophila C virus, Escherichia virus HK022, Formica exsecta virus 1 (FEX-1 / FeV1), Israeli acute paralysis virus (IAPV), **Kashmir bee virus (KBV)***, Linepithema humile bunya-like virus 1, Linepithema humile C-virus 1, Linepithema humile entomopoxvirus 1, Linepithema humile narna-like virus 1, Linepithema humile partiti-like virus 1, Linepithema humile picorna-like virus 1, **Linepithema humile polycipivirus 1***, Linepithema humile polycipivirus 2, Linepithema humile qinvirus-like virus 1, Linepithema humile rhabdo-like virus 1, Linepithema humile toti-like virus 1, **Linepithema humile virus 1***, Linepithema humile virus 2, Moku virus, Pseudomonas phage PS-1, Rhopalosiphum padi virus, Salmonella phage SJ46, Shigella phage SfIV, Solenopsis invicta virus 1 (SINV-1), Sp6virus, Thika virus
Tapinoma melanocephalum	ABPV, BQCV, DWV, IAPV, KBV, Lake Sinai virus (LSV), Milolii virus, Moku virus, Sacbrood virus (SBV), Slow bee paralysis virus (SBPV)
Subfamily - Formicinae	
Anoplolepis gracilipes	TR17983 virus, **TR44839 virus***, TR80102 virus
Brachymyrmex sp.	BQCV, DWV, SBV
Camponotus sp.	ABPV, BQCV, DWV, IAPV, SBV
Camponotus japonicus	Wuhan Ant Virus 1
Camponotus nipponicus	Camponotus nipponicus virus
Camponotus vagus	**Chronic bee paralysis virus (CBPV)***
Camponotus yamaokai	Camponotus yamaokai virus
Colobopsis shohki	Colobopsis shohki virus 1
Formica aquilonia	FEX-1 / FeV1
Formica cinerea	FEX-1 / FeV1, Formica exsecta virus 2 (FeV2), KBV
Formica exsecta	FEX-1 / FeV1, FeV2, Formica exsecta virus 3, Formica exsecta virus 4 (FeV4), KBV
Formica fusca	FEX-1 / FeV1, FeV2, FeV4, **Formica fusca virus 1***, KBV
Formica pressilabris	FEX-1 / FeV1, FeV2, KBV
Formica rufa	CBPV
Formica truncorum	FEX-1 / FeV1, FeV2
Lasius neglectus	Lasius neglectus virus 1, **Lasius neglectus virus 2***
Lasius niger	Lasius niger virus 1, **ABPV***, DWV (strains A and B)
Lasius platythorax	**ABPV***, DWV (strains A and B)
Nylanderia sp.	ABPV, BQCV, IAPV
Nylanderia fulva	**Nylanderia fulva virus 1***
Subfamily - Myrmicinae	
Aphaenogaster texana	ABPV
Crematogaster sp.	ABPV, BQCV, DWV, IAPV, KBV, SBV
Monomorium pharaonis	Monomorium pharaonis virus 1, Monomorium pharaonis virus 2
Myrmica scabrinodis	Myrmica scabrinodis virus 1, **Myrmica scabrinodis virus 2***
Pheidole sp.	ABPV, BQCV, DWV, IAPV

Ant species	Viruses
Pheidole megacephala	ABPV, BQCV, DWV (strains A, B and C), IAPV, KBV, LSV, Milolii virus, Moku virus, SBV, SBPV
Pogonomyrmex sp.	ABPV
Pogonomyrmex californicus	KBV
Solenopsis carolinensis	Solenopsis invicta virus 1A (SINV-1A)
Solenopsis geminata	SINV-1
Solenopsis geminata	SINV-1A
Solenopsis geminata/ xyloni hybrid	SINV-1
Solenopsis invicta	ABPV, Alber virus, Aphid lethal paralysis virus, Autographa californica nuclear polyhedrosis virus (AcNPV) AaIT-p10, AcNPV-AaIT-ie1, AcNPV-LqhIT2-ie1, Big Sioux River virus, BQCV, DWV, Drosophila C virus, Helicoverpa zea nuclear polyhedrosis virus (HzNPV), HzNPV-LqhIT2-ie1, Hubei orthoptera virus 1, Hubei picorna-like virus 46, Hubei picorna-like virus 50, IAPV, KBV, Mosinovirus, Nasonia vitripennis virus, Nodamura virus, Rhopalosiphum padi virus, SBV, Shuangao insect virus 8, Solenopsis invicta densovirus, **SINV-1***, SINV-1A, **Solenopsis invicta virus 2 (SINV-2)***, **Solenopsis invicta virus 3 (SINV-3)***, Solenopsis invicta virus 4 (SINV-4), **Solenopsis invicta virus 5 (SINV-5)***, Solenopsis invicta virus 6 (SINV-6), Solenopsis invicta virus 7 (SINV-7), Solenopsis invicta virus 8 (SINV-8), Solenopsis invicta virus 9 (SINV-9), Solenopsis invicta virus 10 (SINV-10), Solenopsis invicta virus 11 (SINV-11), Solenopsis invicta virus 12 (SINV-12), Solenopsis invicta virus 13 (SINV-13), Solenopsis midden virus, Wuhan arthropod virus 2, Wuhan insect virus 11
Solenopsis richteri	SINV-1
Solenopsis invicta/ richteri hybrid	SINV-1, SINV-1A, **SINV-3***, SINV-3 hybrid
Subfamily - Pseudomyrmecinae	
Pseudomyrmex gracilis	DWV

ant *Solenopsis invicta* due to efforts to determine if ants serve as reservoirs for honey bee viruses and to discover potential biocontrol agents, respectively. A large number of studies focused on these two ant species, therefore, it is highly likely that a substantial diversity of viruses still remains unknown in the other > 13,500 ant species on the planet (Borowiec & al. 2020). Most of the reported viruses belong to the Picornavirales order of single-stranded RNA (ssRNA) viruses. More than half of the viruses are positive-sense ssRNA (+ssRNA) viruses, however other viral genome types have also been found including negative-sense ssRNA (-ssRNA); double-stranded RNA (dsRNA); and double-stranded DNA (dsDNA). Replication of +ssRNA viruses involves production of the negative-sense RNA strand which indicates that the virus is replicating and that the ant serves as a host. We report on 13 +ssRNA viruses known to replicate within ant hosts representing four families (Dicistroviridae, Iflaviridae, Polycipiviridae, and Solinviviridae; Fig. 1) in one viral order (Picornavirales). If we include all viral families detected in ants, not only those shown to replicate, then the viruses reported represent 15 families. We note that these numbers include 40 putative viruses that were only detected in high-throughput sequencing datasets and are sometimes represented by only partial sequences, and also include five bacteriophages (in three different bacteriophage families) that do not directly infect ants but bacteria instead.

Virus bioprospecting in ants has led to unique virus discoveries not detected previously, which now represent new taxa. Recently, two new virus families originated from discoveries in ant hosts. These two families have been accepted and ratified by the International Committee on Taxonomy of Viruses (Olendraite & al. 2017, Brown & al. 2019, Olendraite & al. 2019). The family Polycipiviridae belongs to the order Picornavirales, and is characterised by a clade of arthropod-infecting polycistronic picorna-like viruses. It comprises three genera: Sopolycivirus, Hupolycivirus, and Chipolycivirus (Olendraite & al. 2017, Olendraite & al. 2019). The genus Sopolycivirus appears to be specific for the Formicidae (Olendraite & al. 2017). Another recently discovered viral family, Solinviviridae (Brown & al. 2019) is a family of picorna/calici-like viruses, with two genera: Invictavirus and Nyfulvavirus. Two viral species, Solenopsis invicta virus 3 (SINV-3) and Nylanderia fulva virus 1 (NfV-1), infect ants; however, related unclassified virus sequences have been isolated from a variety of other insects and arthropods (Shi & al. 2016, Valles & al. 2016). It is possible that Solinviviridae forms a sister group to Caliciviridae, though phylogenetic clustering remains inconclusive (Valles & al. 2014a).

Two viruses in fire ants, Solenopsis invicta virus 7 and Solenopsis invicta virus 10 were recently discovered and may represent a unique taxonomic group, as they do not fit within the current viral taxonomic structure

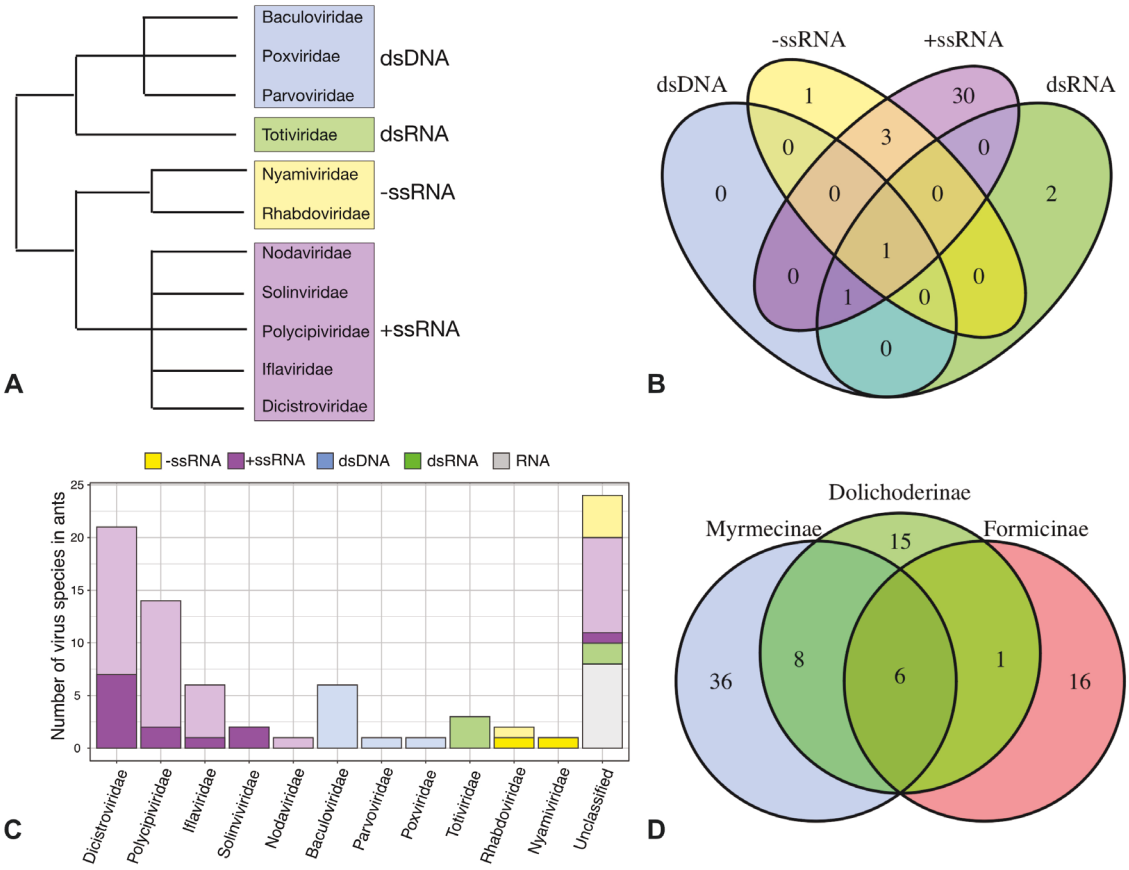

Fig. 1: (A) Tree based on Baltimore classification of viral genome organisation representing the virus families reported in ants (not including bacteriophages). (B) Venn diagram representing the number of ant species infected by different types of viruses (note the overrepresentation of +ssRNA viruses). (C) Barplot showing the number of viruses reported per virus family, including those with evidence for replication in darker colour shade. Evidence of true parasitism or that ants are definitive hosts of the viruses is demonstrated by replication. Only a portion of the viruses found in ants have been shown to replicate. The other viruses may be parasitizing the ants (but we are yet to see evidence of replication) or they may have been ingested in ant prey items. (D) Venn diagram showing the number of virus species detected in three ant subfamilies.

(Valles & Rivers 2019). The same study also identified a Toti-like virus, Solenopsis midden virus, which forms a new monophyletic group divergent from the established Totiviridae genera and appears to infect arthropods exclusively (Valles & Rivers 2019). Another study discovered three ant viruses with sequences highly divergent from previously sequenced viruses in three different ant species, *Formica fusca* Linnaeus, 1758, *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy, 1990, and *Myrmica scabrinodis* Nylander, 1846. Two of the viruses fall within known viral genera, however, the third, Lasius neglectus virus 2, belongs in the family Rhabdoviridae where it groups within an unclassified cluster of rhabdoviruses that infect arthropods (Kleanthous & al. 2019).

Many viruses detected in ants are also found in other species. Out of the 87 viruses reported in ants, 32 are also found in other insects and five are bacteriophages found in various organisms. Only 41 viruses are found in individual ant species and nine in multiple ant species. The five most commonly detected viruses have been described from and are more typically associated with honey bees (*Apis mellifera* Linnaeus, 1758): Acute bee paralysis virus (ABPV, found in 12 ant species); Deformed wing virus (DWV, found in 12 ant species); Kashmir bee virus (KBV, found in 10 ant species); Black queen cell virus (BQCV, found in nine ant species); and Israeli acute paralysis virus (IAPV, found in eight ant species). Honey bees are an economically important species, so have had much focus and examination, allowing for the discovery of these viruses. We suspect that many viruses and pathogens described from honey bees are widely shared amongst hymenopteran hosts (Loope & al. 2019), and may have even evolved in non-bee hosts, but were first discovered and have had the majority of research emphasis in honey bees. For many viruses first isolated in bees, demonstration of replication in ants is still lacking (Fig. 1 and Tab. S1). We recommend testing for viral replication within each ant species in order to be confident that the virus species is parasitizing the ant and that the ant is a true host to the virus. It is also likely that some of the viruses reported in ants may have been present only after prey ingestion. Viral host specificity varies, with some viruses such as SINV-3 being completely

Fig. 2: Ants interact with and prey upon a wide array of insects that host viruses. Here, Argentine ants are raiding a beehive in New Zealand. (A) Honey bees appear to be stressed by ant raids but can do little to deter ants. (B) The ants consume brood and honey, and (C) swarm over emerging bee adults. Some viruses have been found to replicate in bees, Argentine ants and other arthropods (e.g., Deformed wing virus, Kashmir bee virus, Moku virus) (Dobelmann & al. 2020). These and other viruses might be generalist viruses of insects. Other viruses found in honey bees appear to have a more restricted host range (e.g., Black queen cell virus). (Photos by Phil Lester).

specific to ants in the genus *Solenopsis* (Porter & al. 2013, Porter & al. 2015) whereas some other viruses are found in a diversity of arthropods.

Virus transmission

In social species there are direct and indirect opportunities for virus transmission from one individual to another. The transmission potential of a virus increases in association with three factors: abundance (host and viral load), prevalence (number of infected individuals), and infectivity (ability to initiate infection). Where societies interact with other species there is the additional risk of viral transmission to new hosts which can have substantial effects and are a major cause of emerging infectious diseases (Johnson & al. 2015). Of the many viral types, ssRNA viruses are especially capable of switching hosts due to the high mutation rate caused by error-prone RNA-dependent RNA polymerase (reviewed in Holmes 2009). An example is the +ssRNA virus DWV, which has multiple hosts including various ant species, and has become a major cause of honey bee mortality around the globe (Schroeder & Martin 2012).

Viruses can be transmitted horizontally or vertically (Chen & al. 2006, Cremer & al. 2007). Horizontally, viruses are transmitted between individuals in an ecosystem, whereas vertical transmission describes situations when infections spread from mother to offspring. Horizontal transmission of viruses by feeding has been confirmed for viruses in species such as the yellow crazy ant, *Anoplolepis gracilipes* (Smith, 1857) (Hsu & al. 2019a), *Lasius* spp. (Schläppi & al. 2020), and red imported fire ants, *Solenopsis* spp. (Valles & Hashimoto 2009) as discussed in the next paragraph. Horizontal, inter-specific transmission of these viruses is likely to happen via direct or indirect interaction with infected honey bees especially for the variety of ant species found living on or near bee hives (Fig. 2) (Celle & al. 2008, Levitt & al. 2013, Sebastien & al. 2015, Gruber & al. 2017, Brettell & al. 2019, Lester & al. 2019, Dobelmann & al. 2020, Payne & al. 2020, Schläppi & al. 2020). Ants have been observed foraging on virus-infected bees discarded from bee hives and laboratory-based experiments have demonstrated that DWV and ABPV can be transmitted via ingestion by ants consuming homogenates of infected honey bee pupae (Schläppi & al. 2019, Schläppi & al. 2020). Ants interact with bees in other ways that provide opportunities for infection, such as predation; co-habitation; and robbing of hive resources including honey (Payne & al. 2020).

Within ant colonies, horizontal transmission is likely to occur through the many different interactions infected ants have with other colony members. Like other social insects, ants often live in high densities within nests and cooperate for the success of the colony, which carries risk of viral spread. Trophallaxis and allogrooming are examples of close-contact behaviour and possible routes for virus transmission. Solenopsis invicta virus 1, 2 and 3 (SINV-1, SINV-2, SINV-3) all target the midgut, and it has been hypothesized that virus particles can spread from there to the gut lumen, and then to other ants via trophallaxis or by contact with contaminated faeces (Hashimoto & al. 2007, Hashimoto & Valles 2008a, Valles & Hashimoto 2009, Valles 2012). Laboratory and field experiments with various formulations of SINV-3, including crushed-ants in sugar solutions, have demonstrated transmission of this virus via ingestion (Valles & Hashimoto 2009, Porter & al. 2013, Valles & Porter 2013, Valles & Oi 2014, Valles & Porter 2015), and showed the virus could also spread over 100 m from the initial bait station (Oi & al. 2019). *Solenopsis invicta* ants can also become infected with SINV-3 by consuming house crickets *Acheta domesticus* Linnaeus, 1758 that had consumed dead infected ants (Porter & al. 2016b). Solenopsis invicta virus 3 and Nylanderia fulva virus 1 are particularly infectious with unintended virus-transmission occurring in

laboratory-reared ant colonies (Valles & Porter 2013, Valles & al. 2016).

There is some evidence for vertical transmission for a few viruses including SINV-2, which was detected in queens and eggs of *Solenopsis invicta* as well as other developmental stages (Hashimoto & Valles 2008b). Similarly, Camponotus yamaokai virus was detected in all *Camponotus yamaokai* Terayama & Satoh, 1990 caste members and developmental stages including eggs (Koyama & al. 2015). Currently, the prevalence of vertical transmission as a mode of transmission is unknown for most viruses in ants as there have been few studies examining transmission dynamics.

Effects of viruses on ant physiology

The majority of studies on ant viruses have focused on viral molecular characterization, phylogenetics, host specificity, transmission, geographical distribution and genomics with only a limited number of studies reporting on the effects of viruses on ant physiology. Indeed, the best-studied viruses in terms of physiological effects are three viruses infecting ants in the genus *Solenopsis* (Valles & al. 2007b): SINV-1 (Valles & al. 2004); SINV-2 (Valles & al. 2007a); and SINV-3 (Valles & Hashimoto 2009). The effects of these viruses span from slight fitness costs to colony mortality. For the majority of ant viruses, little is known of their pathogenicity possibly because infections remain asymptomatic. We summarised the known effects (or observed absence of effects) for 13 different viruses that attack ants (Tab. 2).

Solenopsis invicta virus 1 appears to affect early stages of a queen's life cycle, from development in her natal colony up to, or shortly after, her nuptial mating flight (Manfredini & al. 2016). Queens infected with SINV-1 have decreased body weight which is likely to reduce the probability of successful colony founding. It is not known whether reduced queen weight is due to the infection itself (for example SINV-1 interfering with metabolism), or whether light-weight queens are the product of colonies with chronic infection where food distribution is less efficient, or whether light-weight queens are more likely to become infected due to a lack of energy reserves (Manfredini & al. 2016). Most SINV-1 infections are chronic and remain asymptomatic but may result in mortality under certain conditions of stress (Valles 2012).

Solenopsis invicta virus 2 infections are also chronic and asymptomatic until infected individuals encounter additional stressors (Hashimoto & Valles 2008b). In contrast to SINV-1, SINV-2 appears to affect later stages of colony founding, with significant reductions in queen fecundity (less brood produced) and other detrimental fitness effects, including longer claustral periods, reduced weight and slower growth of newly-established colonies (Manfredini & al. 2016). This virus is associated with greater changes in global gene expression in the host than SINV-1 and SINV-3 (Manfredini & al. 2016). Gene expression data suggests that queens mount a stronger immune response to SINV-2 than to SINV-1 (Manfredini & al. 2016).

Solenopsis invicta virus 3 infection is consistently associated with significant mortality among *Solenopsis invicta* laboratory colonies (Fig. 3). Once *S. invicta* colonies become infected with SINV-3, the same progression of events unfold beginning with cessation of feeding on

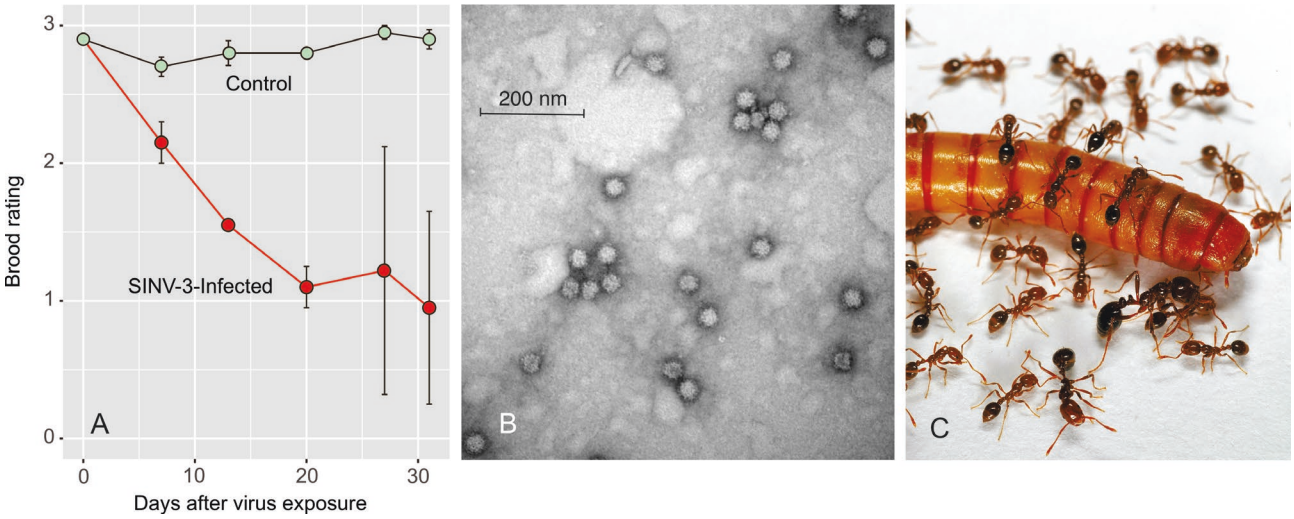

Fig. 3: (A) Solenopsis invicta virus 3 (SINV-3) is pathogenic to infected fire ant colonies. Here we show mean brood ratings (± one standard error) for laboratory colonies of *Solenopsis invicta* infected by SINV-3 compared to uninfected control colonies, adapted from Valles & al. (2014b). The scoring scale ranged from colonies that were without any brood (score = 0), a 2 indicating colonies were in poor health with brood mass of ~ 50% of worker mass, to a 4 which would indicate a rapidly growing colony with brood mass ≥ worker mass. Figure adapted from Valles & al. (2014b), Solenopsis invicta virus 3: Pathogenesis and stage specificity in red imported fire ants, 67, Copyright (2014), with permission from Elsevier. (B) An electron micrograph of a negative stain of a SINV-3 preparation obtained from *Solenopsis invicta* worker ants and purified by cesium chloride isopycnic centrifugation (photo by Steven Valles). (C) A laboratory colony of *S. invicta* attacking a mealworm larva (*Tenebrio molitor*) (photo by Phil Lester).

Tab. 2: Physiological and gene expression effects of viruses (or the reported lack of effects) in ants. Tissue tropism refers to organs in which viruses were detected.

Virus	Tropism	Physiological effects on ants	Physiological effects on other insects
ABPV	In honey bees, ABPV accumulates in the brain and hypopharyngeal glands of adults, and in faeces, semen, and in the brood (de Miranda & al. 2010).	Infected *L. niger* ants present with impaired locomotion, shakiness and uncontrolled movements. Infected colonies were smaller in size as fewer adults emerged (Schläppi & al. 2020).	In honey bees, the death of infected adults is preceded by rapidly progressing paralysis, including trembling and inability to fly. There is gradual darkening and loss of hair from the thorax and abdomen (Bailey & al. 1963, Maori & al. 2007, Ribière & al. 2008). Severe infections result in sharp declines in bee populations with the appearance of diseased larvae and pupae due to the lack of adults available to tend brood (reviewed in de Miranda & al. (2010).
BQCV	Detected in the gut tissue of honey bees (Beaurepaire & al. 2020).	In *L. humile* ants BQCV had the lowest rate of infection or load compared with other studied viruses. Viral load positively correlated with KBV and was not significantly associated with the expression of any immune gene studied (Lester & al. 2019).	In honey bees, queen larvae turn yellowish with sac-appearance, later turning dark brown. Infected pupae turn brown and die. Queen cell walls turn dark brown/black. Adults exhibit shortened lifespan (reviewed in Beaurepaire & al. 2020).
CBPV	In honey bees, this virus exhibits neurotropism, and is detected in the alimentary tract, mandibular and hypopharyngeal glands (Ribière & al. 2010).	In a study of infected *C. vagus* and *F. rufa*, there were no observations of trembling ants in the field, although dead ants were found along with dead bees around apiaries (Celle & al. 2008).	Symptoms in bees include: bloated abdomens; partially-spread dislocated wings trembling motion; inability to fly; and a tendency to crawl on the ground and up plant stems, sometimes in masses of thousands of individuals. Bees often huddle together on top of the cluster in the hive. Distension of the honey sac with fluid leads to 'dysentery'. Sick individuals die within a few days of the onset of symptoms. Infected bees lose hair and appear dark and almost black, shiny and greasy. They suffer nibbling attacks by healthy bees of their colony (appearing like robber bees). Severely infected colonies suddenly collapse, leaving queens with a few workers (Bailey & Ball 1991, Ball & Bailey 1997 and reviewed in Ribière & al. 2010).
DWV	In honey bee queens, DWV concentrates in the reproductive tissues the ovaries and spermatheca but was also directly observed in fat body cells (Fievet & al. 2006).	DWV replicates in *M. rubra* however, colony development showed no abnormalities and worker ants did not show clinical symptoms (Schläppi & al. 2019). Viral loads varied enormously among infected *L. humile* ants, and viral loads were orders of magnitude higher in the presence of honey bees. Levels of infection were an order of magnitude higher than for KBV and BQCV (Lester & al. 2019).	In honey bees, wings are crumpled or aborted, and abdomens are shortened. The lifespan of adult workers and drones is shortened considerably. Individuals show impaired learning and forging behaviour (reviewed in Beaurepaire & al. 2020).
FeV1		No overt symptoms noted in *F. exsecta* (Johansson & al. 2013).	
FeV2		Males and queen *F. exsecta* ants with deformed wings have been observed from sampled populations but the culprit has not been confirmed (Johansson & al. 2013, Dhaygude & al. 2019).	
FeV4		No overt symptoms noted in *F. exsecta* (Johansson & al. 2013).	
KBV	In honey bees, KBV is detected in faeces, surface-sterilised eggs but not in the queen ovaries (de Miranda & al. 2010).	There has been no indication of *L. humile* colony suffering or collapse (Abril & Jurvansuu 2020). In queens from the European supercolony, KBV was the most common virus. A high percentage of queens had unusually high viral loads, indicating active infection (Viljakainen & al. 2018a).	In honeybees, infections are associated with sharp decline in adult bees resulting in diseased larvae and pupae due to the lack of adults available to tend the brood (reviewed in (de Miranda & al. 2010).

Virus	Tropism	Physiological effects on ants	Physiological effects on other insects
LHUV-1		No indication of *L. humile* colony suffering or collapse (Abril & Jurvansuu 2020). However, they mount a substantial immune response to infection, involving almost all immune pathways (Lester & al. 2019). Viral loads varied enormously and were orders of magnitude higher in ants outside of apiaries. Viral load negatively correlated with KBV load.	
NfV-1	Detected in *N. fulva* workers, larvae, pupae, and queens with the exception of eggs (Valles & al. 2016).	Infected *N. fulva* lack overt symptoms. One infected colony in a transmission experiment declined over time exhibiting significant mortality among brood (83%) and workers (76%). However, other infected colonies in the experiment seemed to thrive (Valles & al. 2016).	
SINV-1	Replicates in the midgut epithelial cells of larvae and adults with infectious viral particles are shed into the gut lumen (Oi & Valles 2009, Valles 2012).	Infections persist chronically and asymptomatically. When infected colonies encounter stressors, the virus replicates rapidly with host symptoms and death (Valles 2012). Significant brood mortality has been observed following colony translocation (Valles & al. 2004, Hsu & al. 2019b). SINV-1 appears to play a role in early queen life, from natal colony development up to/shortly after her nuptial mating flight. Infected queens weighed significantly less at colony foundation, likely reducing the probability of successful colony founding (Manfredini & al. 2016). Infections decreased mortality in *S. invicta* exposed to some insecticides through unknown mechanism/s, possibly through decreased foraging activity at toxic baits in infected colonies (Tufts & al. 2014, Hsu & al. 2018).	.
SINV-2	Midgut epithelial cells of adults and larvae	Infections impact later stages of colony founding, resulting in queens with reduced reproductive output. SINV-2 has detrimental effects on fire ant incipient colonies: infected colonies have longer claustral periods, weigh less and grow slowly (Manfredini & al. 2016).	
SINV-3	Detected in all tissues of *S. invicta* queens, workers and larvae (Valles & Hashimoto 2009, Valles 2012).	Severe disease symptoms following infection can lead to colony death (Valles & Hashimoto 2009). Infected colonies exhibit a characteristic disease progression including: cessation of feeding on solid food; massive brood reductions; large numbers of dead ants and brood; queen weight loss and decreased egg production/ovary wasting; and colony collapse in 30-60 days (Porter & al. 2013, Valles & al. 2014b).	

solid food (Chen & al. 2012, Valles & al. 2014b), followed by brood (larval and pupal) mortality (Chen & al. 2012, Porter & al. 2013, Valles & al. 2014b). Early signs of infection include larger than normal midden piles of adult ants followed by near complete brood disappearance (Porter & al. 2013). Larval mortality is thought to occur as a result of starvation or neglect by the worker caste (Valles & al. 2013, Valles & al. 2014b, Valles & Rivers 2019). Queens lose their ability for distension of the abdominal intersegmental membranes (physogastricity) (Valles & Hashimoto 2009). However, they continue to produce eggs. Some workers remain alive for considerable periods after the initial brood die-off, and occasionally the colonies rebound with normal brood production (Valles & Hashimoto 2009, Valles 2012). In later stages of infection, worker mortality increases and queens decrease egg production and lose weight (Valles & al. 2013). The frequency of SINV-3 varies seasonally; its prevalence increases during cooler periods and decreases during warmer periods (Valles & al. 2010). Temperature can affect SINV-3 pathogenicity. Worker ants from SINV-3-treated colonies maintained at low temperatures showed

strong production of the viral capsid protein; indicating that warm summer temperatures combined with fire ant thermoregulatory behaviour might explain lower SINV-3 prevalence during summertime (Valles & Porter 2019). Individual *S. invicta* ants can be infected simultaneously with all combinations of SINV-1, SINV-2 and SINV-3 (Valles & al. 2009, Allen & al. 2011).

There are few studies of the physiological effects of viruses on other ant species. Recently, clinical symptoms of ABPV were found at the individual and colony levels of *Lasius niger* (Linnaeus, 1758) ants (Schläppi & al. 2020). Ants were fed bees infected with two viruses, DWV and ABPV, but only ABPV was found to replicate in these ants. Forager ants from ABPV-fed colonies presented with impaired locomotion and were shaky with uncontrolled movements. No effects of ABPV on body weight were detected, either for queens or workers. However, infected colonies were smaller due to having fewer emerging adults. The impaired movement capabilities and decreased colony size due to ABPV infections are relevant for ant fitness, and may contribute to the weakening of colonies (Schläppi & al. 2020). A larger workforce means a more productive colony, and it also provides an advantage in interspecific conflicts. Larger colonies can be expected to have a higher fitness, as they start reproducing earlier with more gynes and males produced.

Molecular work in Argentine ants examined expression patterns of immune genes in ant communities naturally infected with varying viral species and infection loads. Simultaneous viral and bacterial pathogen infections altered gene expression with both up- and down-regulation of different genes (Viljakainen & al. 2018b). Another study revealed that Argentine ants mounted greater immune responses to the bacteria *Pseudomonas* spp. and Linepithema humile virus 1 (LHUV-1) infections, while BQCV was not associated with strong alteration of immune gene expression (Lester & al. 2019). All immune pathways examined (namely the Jak / STAT, RNAi, Toll, Imd, and JNK pathways) were associated with -ssRNA viruses. The same set of genes were down-regulated in response to dsRNA and most +ssRNA viruses. Surprisingly, genes within the RNAi and Jak / STAT pathways, which are typically associated with anti-viral immune defence, were negatively associated with +ssRNA and dsRNA viruses in Argentine ants. Different microbial pathogens were clearly associated with different immune responses. Patterns of expression for nearly all the immune genes were correlated both within and between immune pathways. The expression levels of the RNAi-pathway genes *Dicer* and *argonaute* were expected to be highly correlated given their involvement in the same anti-viral response. However, nearly all other genes associated with the immune pathways studied were also significantly and highly correlated with these RNAi-pathway genes (Lester & al. 2019).

Viral infections that change ant behaviour

Behavioural changes associated with viral infections in ants can stem from direct pathogenic effects of the virus as well as prophylactic immune defence mechanisms (Cremer & al. 2007). In *Solenopsis invicta*, viral infections have been shown to be associated with changes in foraging behaviour and food collection patterns. Colony-level foraging activity was shown to decrease after inoculation with SINV-1 (Hsu & al. 2018). Infected colonies also displayed changes in diet preferences, with a decline of lipid intake and increased bias towards carbohydrate-rich food. Similarly, SINV-3 decreases protein intake, likely translating into poorer colony health and productivity (Valles & al. 2013, Valles & al. 2014b). Solenopsis invicta virus 3-infected queens suffer from malnutrition and their ovaries are devoid of developing eggs (Valles & al. 2013). The changes in nutrient intake could be associated with "illness-induced anorexia", a defence mechanism believed to have evolved to deprive pathogens from essential macronutrients and allow the allocation of resources towards the immune response instead of digestion (Adamo & al. 2010, Mason & al. 2014). An alternative hypothesis is that infected individuals feeding preference tilts towards carbohydrate-rich food as a compensatory feeding mechanism (Shikano & Cory 2016). Solenopsis invicta virus 1 also decreases competitive abilities of *S. invicta* in staged group and individual interspecific competition assays (Chen & al. 2011). Interestingly, infected colonies did not engage in fights as often as uninfected colonies. A proposed mechanism was the consequence of decreased foraging activity in infected colonies, resulting in lower recruitment.

Aggression has been correlated with viral presence in another invasive ant species, the yellow crazy ant, *Anoplolepis gracilipes*. A virus putatively described as TR44839 was observed in invaded areas of Australia (Cooling & al. 2016). Hsu & al. (2019a) suggest that intercolonial aggressive behaviour in *A. gracilipes* was correlated with virus prevalence. They suggested that strong links between colonies with weak social boundaries (simple colony composition) resulted in epidemics of the TR44839 virus among the colonies, likely induced by horizontal transmission of viruses (Hsu & al. 2019a). Similarly, in red imported fire ants, polygynous colonies harbor a higher diversity of parasites and pathogens when compared to their monogyne conspecifics (Valles & al. 2010). Resource exchanges between non-aggressive colonies are likely to facilitate viral transmission.

Researchers are only beginning to uncover the effects of viruses on ant behaviour and physiology. Virus-associated behavioural changes have been documented in a wide range of insects, including other hymenopterans (Han & al. 2015). In honey bees, the *Kakugo* variant of DWV induces increased aggression, while other DWV strains and IAPV are associated with learning disabilities and impaired foraging (Fujiyuki & al. 2004, 2005, Iqbal & Mueller 2007, Li & al. 2013). While most changes in ant behaviour associated with viral infections are reported for foraging activity, it is likely that viruses affect a wider range of behaviours. A notable example of insect behavioural manipulation by a virus is the parasitoidism of ladybugs, *Coccinella septempunctata* Linnaeus, 1758, by braconid wasps,

Dinocampus coccinellae (Schrank, 1802) (Dheilly & al. 2015). The Dinocampus coccinellae paralysis virus is injected by the ovipositing female adult wasp together with the egg, and migrates into the host's brain, presumably altering the ladybug's behaviour. Fungal, helminth and insect parasites in ants can actively manipulate host behaviour to maximise their own fitness (de Bekker & al. 2018). Although viral manipulation of behaviour has not yet been observed in ants, these examples underline the possibility of virus-mediated behavioural manipulation.

Studies specifically related to viral infections are scarce, but there is a large number of studies investigating behavioural defence mechanisms against entomopathogenic fungi. A number of these reveal mechanisms that are proactive and function as prophylaxis, for example: the division of labour between individuals resulting in differences in exposure to pathogens; the use of antimicrobial compounds; or waste and corpse management (Schmid-Hempel 1998, Chapuisat & al. 2007, Brütsch & al. 2017, Kesäniemi & al. 2019). However, ant colonies can also adjust their behaviour reactively as a result of active infections. Social interactions within colonies have been shown to undergo changes after experimental infection, suggesting that network plasticity might be a component of disease management (Stroeymeyt & al. 2018). Remarkably, sanitary care between nestmates can also be adjusted depending on individual infection levels, likely resulting in healthier colonies (Konrad & al. 2018). Such behavioural immune defences have been characterised in response to non-viral pathogens and research is needed to elucidate whether virus can also elicit similar responses.

Population dynamics and the potential for biological control using viruses

Boom-and-bust dynamics have been observed in some invasive ant populations prompting the hypothesis that pathogens such as viruses may play a role in regulating ant populations (Lester & Gruber 2016). Low genetic diversity, high abundance and super-colonial behaviour would seem likely to make invasive ants highly susceptible to pathogens. Viruses are known to regulate insect populations and are used in biological control programmes. Double-stranded DNA viruses of the family Baculoviridae are the most common viruses employed for biological control because they can be mass-produced, are generally accepted as safe to humans, highly pathogenic, and can be readily formulated and applied (Lacey & al. 2015). Twenty-six different baculoviruses have been used for biological control of insect pests. In addition to baculoviruses, insect biocontrol has been attempted with two dsDNA viruses in the Parvoviridae and Nudiviridae families, respectively and with one dsRNA virus in the Reoviridae family (Lacey & al. 2015).

Much of our current knowledge of viruses in ant populations is derived from the search for biological control agents for invasive ant species (de Bekker & al. 2018), specifically with red imported fire ants and also due to efforts to determine if Argentine ants serve as reservoirs for honey bee viruses. A total of 41 viruses have been reported in red imported fire ants and 30 in Argentine ants (Tab. 1 and Tab. S1). The diversity of viruses is typically higher in the native range of both ants relative to their invaded range (Valles 2012, Felden & al. 2019, Valles & Rivers 2019). This loss of potential pathogens between natural and invaded areas is a prediction of the enemy release hypothesis (Keane & Crawley 2002) and is a likely explanation as to why invasive ants frequently attain high densities in their invaded range (Yang & al. 2010). The relatively lower expression of several immune pathways primarily targeting viruses in the invaded range appears to support a lower viral pressure that may allow reallocation of resources away from immunity to other functions that result in an increase of the invader's fitness (Felden & al. 2019). Viral diversity appears similarly lower in the introduced range of fire ants but interestingly prevalence of SINV-1, SINV-2 and SINV-3 is higher than in the native range (Yang & al. 2010). More data on virus prevalence between the native and introduced ranges of invasive ants is needed to further relate invasion success to release from natural enemies.

Most currently known viruses appear to typically have subtle effects on the population dynamics of invasive ants. The viruses often appear to be "covert", possibly because of the hygienic behaviour of ant colonies to remove diseased or dead individuals. Viruses such as SINV-1 and SINV-2 have been considered to conform to the paradigm of many arthropod-infecting +ssRNA viruses, in that they typically persist as chronic but asymptomatic infections that do not cause any overt signs or symptoms of disease (Valles 2012). However, both SINV-1 and SINV-2 can lead to ant mortality when the colony is exposed to certain stressors (Oi & al. 2015). For example, infection by SINV-1 can make fire ants more susceptible to attack by other co-occurring ant species (Chen & al. 2011). We are yet to see any colony level effects of any of the viruses discovered in Argentine ants (Sebastien & al. 2015, Gruber & al. 2017, Viljakainen & al. 2018a, Abril & Jurvansuu 2020) or species such as the tawny crazy ant, *Nylanderia fulva* (Mayr, 1862) (Valles & al. 2016).

Of all the viruses infecting ants, SINV-3 has received the most focus as a potential biological control agent. Multiple studies have demonstrated effective SINV-3 transmission in field colonies of *Solenopsis invicta*, and to our knowledge it is the only virus so far used in attempted biological control programme against an invasive ant (Valles & Oi 2014, Oi & al. 2019). The combined high host specificity and high virulence have led researchers to suggest SINV-3 has the potential to be a widespread self-sustaining biocontrol agent or biopesticide (Porter & al. 2013, Porter & al. 2015, Porter & al. 2016a). The dose required to initiate a lethal response in fire ant colonies has been established. Exposure to a SINV-3 bait solution of 10^5 genome equivalents per µL is sufficient to initiate and sustain an infection (Valles & Porter 2015). However, in order to cause lethal colony effects, a SINV-3 bait solution of at least 10^9 genome equivalents per µL appears to be required. Improved efficacy might be

reached by adding more attractive bait carriers, synergists and multiple viruses (Valles & Porter 2015). Solenopsis invicta virus 3 appears highly infectious and readily transmittable between colonies (Valles & Hashimoto 2009, Valles & al. 2014b). Valles & al. (2014b) describe how SINV-3 infections appear to prevent or slow workers from acquiring and distributing solid food to the larvae and queen. Starvation can then occur for both the queen and larvae (Valles & al. 2014b). This process of colony-collapse by starvation and mortality may then take one to two months. Its effects on ant colonies, however, are both temperature- and dose-dependent as described above (Valles & Porter 2015, 2019). This dependency suggests that SINV-3 will not be uniformly effective everywhere, which may help explain the lack of population suppression after introduction to California for biological control (Oi & al. 2019). Furthermore, such biological control option might also be detrimental to the efficiency of bait-formulated pesticide treatments if used in combination, as foraging and food intake is known to decrease with infection with viruses such as SINV-1 (Tufts & al. 2014, Hsu & al. 2018).

A virus (or pathogen) effective as biological control agent in social insects is not necessarily the most virulent, as time is necessary for the virus to infect all members in a colony. Solenopsis invicta virus 3 has a much greater pathogenicity than SINV-1 or SINV-2 (Oi & al. 2019), however, its pathogenicity is lower than that of the many baculoviruses commercially-produced for biological control. For example, the one- to two-month period for fire ant colony mortality by SINV-3 (Valles & al. 2014b, Valles & Porter 2015) contrasts with a LT_{50} (time to kill 50% of a population) of 64 hours for cotton bollworm treated with the commercially produced Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus (Chen & al. 2000).

Highly virulent pathogens likely occur within the native range of many invasive ants. A key problem with the discovery and identification of highly lethal viruses is that a high pathogenicity will likely lead to the elimination of virulent pathogens during introduction events because of strong selection against founders (Yang & al. 2010). This concept is supported by surveys indicating that there are many more viruses in the native range of ants than in invaded countries typically (Felden & al. 2019, Valles & Rivers 2019). Much of the work on viral discovery in invasive ants, including ours, has involved pathogen discovery in the invaded range. We suggest that future work into viruses and other biological control agents for invasive ants involve the collection and rearing of many different colonies within the home range of the invasive ants. There are surveys that involve extensive sampling for viral and other pathogens within the native range of invasive ants (Valles & al. 2018), but the sampled ants are typically killed and transported internationally for analysis. Transportation itself can be a viral and survival stressor (Sakuna & al. 2017) that could trigger mass viral replication causing significant mortality in ant colonies. The focus on virus and pathogen discovery should be on colonies that experience high mortality due to highly transmittable pathogens which satisfy Koch's postulates for sequence-based pathogen identification (Fredericks & Relman 1996). The efficiency of this work could or should be facilitated by a multi-national approach, ideally with different countries that harbour invasive ants providing resources to sustain pathogen discovery facilities in an area that encompasses the home-range of multiple invasive ants, such as Argentina. Multiple invasive ant species could be simultaneously examined and a sample bank created for future analysis.

Equally critical for biological control of invasive ants is the ability to rear and mass-produce any viral pathogen that is discovered. It has been previously recognised that a major constraint of the use of RNA viruses is a process for their large-scale production and lack of resources such as cell lines (Valles 2012, Oi & al. 2015). Methods for the development of a cell line from embryonic honey bee tissues could be adapted for ants (Goblirsch & al. 2013). An alternative option to propagating viruses in vitro is to utilize a baculovirus expression system. Allen & Valles (2015) utilised an insect cell line, *Spodoptera frugiperda* 21 that allowed production of a baculovirus expressing full-length SINV-3 transcripts. Unfortunately, despite successful transcription, in this case there was no evidence of translation (Allen & Valles 2015), although it may be a viable option for the expression of other viruses.

Methodology for viral discovery and delimitation in ant communities

Various approaches are employed to detect viruses in ants including: reverse transcription PCR (RT-PCR); Sanger sequencing; Next Generation Sequencing (NGS); and Rapid amplification of cDNA ends (RACE) (Tab. S1). High-throughput sequencing has revolutionised the discovery and survey of viruses. Unlike PCR-based methods, these methods do not require specific knowledge of viral genomes. Hence, recent studies have reported unprecedented numbers of new viruses using RNA high-throughput sequencing techniques (Shi & al. 2016, Gruber & al. 2017, Viljakainen & al. 2018a). Data for the study of viruses may come from messenger RNA (mRNA) sequencing (RNA-seq) or microRNA (miRNA) sequencing (sRNA-seq), as these techniques allow both for detection of RNA viruses as well as actively replicating DNA viruses. An advantage of high-throughput RNA sequencing techniques resides in the ability to characterise virus presence and loads as well as the immune response of the host. In RNA-seq studies, data allow for the analysis of immune gene expression possibly related to the host's response against viruses (Lester & al. 2019). In sRNA-seq experiments, the sequencing of short RNA provides information on both viral infection and also on the host RNA interference (RNAi) immune response via the production of virus-specific miRNA (Wu & al. 2010, Viljakainen & Jurvansuu 2020). Published RNA-seq databases from studies focusing on ant gene expression may harbour

known and even unknown viruses in reads that did not map to host genomes and are typically discarded from gene expression studies.

PCR-based methods remain extremely useful to confirm entire viral genomes and are more relevant in surveys of specific, already characterised viruses. Furthermore, PCR-based methods also allow investigation of viral replication, which is an important component of pathogen dynamics. Assays such as RT-PCR and quantitative RT-PCR are commonly used to confirm virus presence when coverage in high-throughput sequencing is low or to test for load or prevalence of specific viruses in different tissues or hosts. Quantitative RT-PCR can provide indirect evidence for active virus infections when viral genome concentrations increase in repeated sampling. Yet, in some cases viral loads may increase without active viral replication. Trophallaxis, the feeding behaviour in which fluids are shared between different members of an ant society, caused elevated SINV-3 levels in larvae of infected *Solenopsis invicta* colonies (Valles & Porter 2013), however, the lack of negative viral strands, small genome fragments (miRNA) and viral capsid protein VP2 production in larvae later revealed that SINV-3 replication is limited to the adult stage from which the larvae acquired increased SINV-3 concentrations (Valles & al. 2014b). Methods used to test for active viral infections include northern blot for miRNA detection, western blot to detection viral capsid proteins and strand specific RT-PCR to detect the negative strand intermediate that +ssRNA viruses produce during replication (Valles & al. 2014b).

To date, only 13 +ssRNA viruses have been shown to replicate in ants using strand-specific RT-PCR (Tab. S1). This common tool is used to confirm active viral infections and is usually based on a protocol first developed by Craggs & al. (2001). During the RT step cDNA specific to the negative (antisense) strand is generated by using a virus-specific primer tagged with an unrelated sequence. To improve specificity and avoid false-positives, many studies additionally use enzymes to digest remaining RNA and single-stranded DNA (such as unincorporated RT-primers) before the PCR step. The PCR is then carried out in presence of primers specific to the virus and the tag sequence so that only cDNA derived from the tagged RT-PCR primer will be amplified (Craggs & al. 2001). This method is considered the gold standard of detecting replicative forms of +ssRNA viruses and establishing host status. For instance, it was used to show that SINV-3 only replicates in the *Solenopsis saevissima* (Smith, 1855) complex of fire ants and not in other ant species exposed to the virus (Porter & al. 2013). The host range of bee-infecting viruses has increasingly gained interest as emerging viral diseases may pose a significant risk to pollinator health. Nineteen different ant species have been found to be positive for viruses that are known to infect honey bees (Tab. 1) (Celle & al. 2008, Levitt & al. 2013, Gruber & al. 2017, Brettell & al. 2019, Lester & al. 2019, Dobelmann & al. 2020, Payne & al. 2020), raising concerns about virus reservoirs in ant populations (Sebastien & al. 2015). However, strand-specific RT-PCR has shown that only four ant species display active replication of bee-infecting viruses, including DWV and KBV replication in *Linepithema humile* (see Gruber & al. 2017), CBPV replication in *Camponotus vagus* (Scopoli, 1763) (Celle & al. 2008) and ABPV replication in *Lasius niger* and *Lasius platythorax* Seifert, 1991 (Schläppi & al. 2020). These findings suggest that RT-PCR may be prone to detecting viruses that are not actively infecting the ant host. Viruses are equipped with a variety of strategies to protect viral RNA from degradation (Dickson & Wilusz 2011), perhaps causing positive RT-PCR results when ants forage on infected bees or ingest other virus-contaminated material. Research on DWV in the ectoparasitic mite *Varroa destructor* Anderson & Trueman, 2000 shows that even negative strand intermediates from ingested honey bee tissue can be detected although the virus does not actively replicate within mites (Posada-Florez & al. 2019). Quantification of negative- and positive-strand copies has been used to study replication efficiency in different life stages of fire ants (Hashimoto & Valles 2008a). Quantitative strand-specific RT-PCR could help to resolve the question of active virus replication in different hosts, which can serve as an indicator of disease severity (Valles & Porter 2015) that can improve understanding pathogenicity of ant viruses.

Concluding remarks

Further studies are clearly warranted to understand how viral infections affect ant physiology and behaviour at the individual and colony level. It is evident that the more we look, the more we find, both in terms of viral discovery and viral impacts on their ant hosts. Rates of viral discovery in ants will increase in the following decades with new and relatively cheaper techniques. Given the recent discovery and description of a diverse community of bacteriophage viruses in the gut bacterial communities of honey bees (Bonilla-Rosso & al. 2020, Deboutte & al. 2020), it seems likely that the diverse community of gut bacteria in ants will also be subject to a wide array of bacteriophages. Similarly, the recent description of how viruses can dramatically alter ant behaviour suggests that viruses may commonly affect individual hosts and colony dynamics (Hsu & al. 2019a, Schläppi & al. 2020). Multiple pathogens also share ant hosts. It has been hypothesised that within ants, different pathogens could compete for host resources or might be synergistic in their effects on ant physiology (Lester & al. 2019).

The challenge remains to find viruses that are pathogenic enough to be utilized for biological control of invasive ants. The majority of viral infections that have been studied are likely those having sub-lethal or mild effects on their ant hosts. These sub-lethal viral infections are much more likely to be moved to a new geographic range and discovered in any survey, compared to viruses that quickly kill a colony. Because of their nature, the more damaging viruses are largely understudied as acutely-infected colonies most likely died before sampling. We highly

recommend more in situ studies of viral infections on ants in their native range. In situ rearing and monitoring of large numbers of colonies to study their viruses would increase chances of finding viruses more useful for biological control.

Acknowledgements

We thank Laura Brettell for unpublished data on ant viral diversity, Steven Valles and an anonymous referee for useful comments that substantially improved our manuscript.

References

Abril, S. & Jurvansuu, J. 2020: Season- and caste-specific variation in RNA viruses in the invasive Argentine ant European supercolony. – Journal of General Virology 101: 322-333.

Adamo, S.A., Bartlett, A., Le, J., Spencer, N. & Sullivan, K. 2010: Illness-induced anorexia may reduce trade-offs between digestion and immune function. – Animal Behaviour 79: 3-10.

Allen, C. & Valles, S.M. 2015: Successful transcription but not translation or assembly of *Solenopsis invicta* virus 3 in a baculovirus-driven expression system. – Florida Entomologist 98: 943-949.

Allen, C., Valles, S.M. & Strong, C.A. 2011: Multiple virus infections occur in individual polygyne and monogyne *Solenopsis invicta* ants. – Journal of Invertebrate Pathology 107: 107-111.

Avery, S.W., Jouvenaz, D.P., Banks, W.A. & Anthony, D.W. 1977: Virus-like particles in a fire ant, *Solenopsis* sp., (Hymenoptera: Formicidae) from Brazil. – Florida Entomologist 60: 17-20.

Bailey, L. & Ball, B.V. 1991: Honey bee pathology. – Harcourt Brace Jovanovich, Sidcup, UK, 208 pp.

Bailey, L., Gibbs, A.J. & Woods, R.D. 1963: Two viruses from adult honey bees (*Apis mellifera* Linnaeus). – Virology 21: 390-395.

Ball, B.V. & Bailey, L. 1997: Viruses. In: Morse, R.A. & Flottum, K. (Eds.): Honey bee pests, predators & diseases. – A.I. Root Company, Medina, pp. 11-32.

Beaurepaire, A., Piot, N., Doublet, V., Antunez, K., Campbell, E., Chantawannakul, P., Chejanovsky, N., Gajda, A., Heerman, M., Panziera, D., Smagghe, G., Yañez, O., de Miranda, J.R. & Dalmon, A. 2020: Diversity and global distribution of viruses of the western honey bee, *Apis mellifera*. – Insects 11: art. 239.

Bonilla-Rosso, G., Steiner, T., Wichmann, F., Bexkens, E. & Engel, P. 2020: Honey bees harbor a diverse gut virome engaging in nested strain-level interactions with the microbiota. – Proceedings of the National Academy of Sciences of the United States of America 117: 7355-7362.

Borowiec, M.L., Moreau, C.S. & Rabeling, C. 2020: Ants: phylogeny and classification. In: Starr, C.K. (Ed.): Encyclopedia of social insects. – Springer International Publishing, Cham, pp. 1-18.

Brettell, L.E., Schroeder, D.C. & Martin, S.J. 2019: RNAseq analysis reveals virus diversity within Hawaiian apiary insect communities. – Viruses 11: art. 397.

Brown, K., Olendraite, I., Valles, S.M., Firth, A.E., Chen, Y., Guérin, D.M.A., Hashimoto, Y., Herrero, S., de Miranda, J.R. & Ryabov, E.V. 2019: ICTV Virus taxonomy profile: Solinviviridae. – Journal of General Virology 100: 736-737.

Brütsch, T., Jaffuel, G., Vallat, A., Turlings, T.C.J. & Chapuisat, M. 2017: Wood ants produce a potent antimicrobial agent by applying formic acid on tree-collected resin. – Ecology and Evolution: 2249-2254.

Celle, O., Blanchard, P., Olivier, V., Schurr, F., Cougoule, N., Faucon, J.-P. & Ribière, M. 2008: Detection of chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. – Virus Research 133: 280-284.

Chapuisat, M., Oppliger, A., Magliano, P. & Christe, P. 2007: Wood ants use resin to protect themselves against pathogens. – Proceedings of The Royal Society B-Biological Sciences 274: 2013-2017.

Chen, X., Sun, X., Hu, Z., Li, M., O'Reilly, D.R., Zuidema, D. & Vlak, J.M. 2000: Genetic engineering of *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus as an improved pesticide. – Journal of Invertebrate Pathology 76: 140-146.

Chen, Y., Becnel, J.J. & Valles, S.M. 2012: RNA viruses infecting pest insects. In: Vega, F. & Kaya, H.K. (Eds.): Insect pathology. 2nd edition. – Elsevier, Amsterdam, 133-170 pp.

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

Chen, Y.C., Kafle, L. & Shih, C.J. 2011: Interspecific competition between *Solenopsis invicta* and two native ant species, *Pheidole fervens* and *Monomorium chinense*. – Journal of Economic Entomology 104: 614-621.

Cooling, M., Gruber, M.A.M., Hoffmann, B.D., Sébastien, A. & Lester, P.J. 2016: A metatranscriptomic survey of the invasive yellow crazy ant, *Anoplolepis gracilipes*, identifies several potential viral and bacterial pathogens and mutualists. – Insectes Sociaux 64: 197-207.

Craggs, J.K., Ball, J.K., Thomson, B.J., Irving, W.L. & Grabowska, A.M. 2001: Development of a strand-specific RT-PCR based assay to detect the replicative form of hepatitis C virus RNA. – Journal of Virological Methods 94: 111-120.

Cremer, S., Armitage, S.A. & Schmid-Hempel, P. 2007: Social immunity. – Current Biology 17: R693-R702.

de Bekker, C., Will, I., Das, B. & Adams, R.M.M. 2018: The ants (Hymenoptera: Formicidae) and their parasites: effects of parasitic manipulations and host responses on ant behavioral ecology. – Myrmecological News 28: 1-24.

de Miranda, J.R., Cordoni, G. & Budge, G. 2010: The acute bee paralysis virus-Kashmir bee virus-Israeli acute paralysis virus complex. – Journal of Invertebrate Pathology 103: S30-S47.

Deboutte, W., Beller, L., Yinda, C.K., Maes, P., de Graaf, D.C. & Matthijnssens, J. 2020: Honey-bee-associated prokaryotic viral communities reveal wide viral diversity and a profound metabolic coding potential. – Proceedings of the National Academy of Sciences of the United States of America 117: 10511-10519.

Dhaygude, K., Johansson, H., Kulmuni, J. & Sundstrom, L. 2019: Genome organization and molecular characterization of the three *Formica exsecta* viruses-FeV1, FeV2 and FeV4. – PeerJ 6: art. e6216.

Dheilly, N.M., Maure, F., Ravallec, M., Galinier, R., Doyon, J., Duval, D., Leger, L., Volkoff, A.N., Missé, D., Nidelet, S., Demolombe, V., Brodeur, J., Gourbal, B., Thomas, F. & Mitta, G. 2015: Who is the puppet master? Replication of a parasitic wasp-associated virus correlates with host behaviour manipulation. – Proceedings of the Royal Society B-Biological Sciences 282: art. 20142773.

Dickson, A.M. & Wilusz, J. 2011: Strategies for viral RNA stability: live long and prosper. – Trends in Genetics 27: 286-293.

Dobelmann, J., Felden, A. & Lester, P.J. 2020: Genetic strain diversity of multi-host RNA viruses that infect a wide range of pollinators and associates is shaped by geographic origins. – Viruses 12: art. 358.

Felden, A., Paris, C., Chapple, D.G., Suarez, A.V., Tsutsui, N.D., Lester, P.J. & Gruber, M.A.M. 2019: Native and introduced Argentine ant populations are characterised by distinct transcriptomic signatures associated with behaviour and immunity. – NeoBiota 49: 105-126.

Fievet, J., Tentcheva, D., Gauthier, L., de Miranda, J.R., Cousserans, F., Colin, M.E. & Bergoin, M. 2006: Localization of deformed wing virus infection in queen and drone *Apis mellifera* L. – Virology Journal 3: art. 16.

Fredericks, D.N. & Relman, D.A. 1996: Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. – Clinical Microbiology Reviews 9: 18-33.

Fujiyuki, T., Takeuchi, H., Ono, M., Ohka, S., Sasaki, T., Nomoto, A. & Kubo, T. 2004: Novel insect picorna-like virus identified in the brains of aggressive worker honeybees. – Journal of Virology 78: 1093-1100.

Fujiyuki, T., Takeuchi, H., Ono, M., Ohka, S., Sasaki, T., Nomoto, A. & Kubo, T. 2005: Kakugo virus from brains of aggressive worker honeybees. – Advances in Virus Research 65: 1-27.

Goblirsch, M.J., Spivak, M.S. & Kurtti, T.J. 2013: A cell line resource derived from honey bee (*Apis mellifera*) embryonic tissues. – Public Library of Science One 8: art. e69831.

Gruber, M.A.M., Cooling, M., Baty, J.W., Buckley, K., Friedlander, A., Quinn, O., Russell, J., Sebastien, A. & Lester, P.J. 2017: Single-stranded RNA viruses infecting the invasive Argentine ant, *Linepithema humile*. – Scientific Reports 7: art. 3304.

Han, Y., van Oers, M.M., van Houte, S. & Ros, V.I.D. 2015: Virus-induced behavioural changes in insects. In: Mehlhorn, H. (Ed.): Host manipulations by parasites and viruses. – Springer International Publishing, Cham, pp. 149-174.

Hashimoto, Y. & Valles, S.M. 2008a: Detection and quantitation of Solenopsis invicta virus-2 genomic and intermediary replicating viral RNA in fire ant workers and larvae. – Journal of Invertebrate Pathology 98: 243-245.

Hashimoto, Y. & Valles, S.M. 2008b: Infection characteristics of Solenopsis invicta virus 2 in the red imported fire ant, *Solenopsis invicta*. – Journal of Invertebrate Pathology 99: 136-140.

Hashimoto, Y., Valles, S.M. & Strong, C.A. 2007: Detection and quantitation of *Solenopsis invicta* virus in fire ants by real-time PCR. – Journal of Virological Methods 140: 132-139.

Holmes, E.C. 2009: The evolutionary genetics of emerging viruses. – Annual Review of Ecology, Evolution, and Systematics 40: 353-372.

Hsu, H.W., Chiu, M.C., Lee, C.C., Lee, C.Y. & Yang, C.S. 2019a: The association between virus prevalence and intercolonial aggression levels in the yellow crazy ant, *Anoplolepis gracilipes* (Jerdon). – Insects 10: art. 436.

Hsu, H.W., Chiu, M.C., Shih, C.J., Matsuura, K. & Yang, C.S. 2019b: Apoptosis as a primary defense mechanism in response to viral infection in invasive fire ant *Solenopsis invicta*. – Virology 531: 255-259.

Hsu, H.W., Chiu, M.C., Shoemaker, D. & Yang, C.S. 2018: Viral infections in fire ants lead to reduced foraging activity and dietary changes. – Scientific Reports 8: art. 13498.

Iqbal, J. & Mueller, U. 2007: Virus infection causes specific learning deficits in honeybee foragers. – Proceedings of the Royal Society B-Biological Sciences 274: 1517-1521.

Johansson, H., Dhaygude, K., Lindstrom, S., Helanterä, H., Sundstrom, L. & Trontti, K. 2013: A metatranscriptomic approach to the identification of microbiota associated with the ant *Formica exsecta*. – Public Library of Science One 8: art. e79777.

Johnson, P.T.J., de Roode, J.C. & Fenton, A. 2015: Why infectious disease research needs community ecology. – Science 349: art. 1259504.

Keane, R. & Crawley, M.J. 2002: Exotic plant invasions and the enemy release hypothesis. – Trends in Ecology & Evolution 17: 164-170.

Kesäniemi, J., Koskimäki, J.J. & Jurvansuu, J. 2019: Corpse management of the invasive Argentine ant inhibits growth of pathogenic fungi. – Scientific Reports 9 art. 7593.

Kleanthous, E., Olendraite, I., Lukhovitskaya, N.I. & Firth, A.E. 2019: Discovery of three RNA viruses using ant transcriptomic datasets. – Archives of Virology 164: 643-647.

Konrad, M., Pull, C.D., Metzler, S., Seif, K., Naderlinger, E., Grasse, A.V. & Cremer, S. 2018: Ants avoid superinfections by performing risk-adjusted sanitary care. – Proceedings of the National Academy of Sciences of the United States of America 115: 2782-2787.

Koonin, E.V. & Dolja, V.V. 2013: A virocentric perspective on the evolution of life. – Current Opinion in Virology 3: 546-557.

Koyama, S., Urayama, S., Ohmatsu, T., Sassa, Y., Sakai, C., Takata, M., Hayashi, S., Nagai, M., Furuya, T., Moriyama, H., Satoh, T., Ono, S. & Mizutani, T. 2015: Identification, characterization and full-length sequence analysis of a novel dsRNA virus isolated from the arboreal ant *Camponotus yamaokai*. – Journal of General Virology 96: 1930-1937.

Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M. & Goettel, M.S. 2015: Insect pathogens as biological control agents: back to the future. – Journal of Invertebrate Pathology 132: 1-41.

Lester, P.J., Buick, K.H., Baty, J.W., Felden, A. & Haywood, J. 2019: Different bacterial and viral pathogens trigger distinct immune responses in a globally invasive ant. – Scientific Reports 9: art. 5780.

Lester, P.J. & Gruber, M.A.M. 2016: Booms, busts and population collapses in invasive ants. – Biological Invasions 18: 3091-3101.

Levitt, A.L., Singh, R., Cox-Foster, D.L., Rajotte, E., Hoover, K., Ostiguy, N. & Holmes, E.C. 2013: Cross-species transmission of honey bee viruses in associated arthropods. – Virus Research 176: 232-240.

Li, Z., Chen, Y., Zhang, S., Chen, S., Li, W., Yan, L., Shi, L., Wu, L., Sohr, A. & Su, S. 2013: Viral infection affects sucrose responsiveness and homing ability of forager honey bees, *Apis mellifera* L. – Public Library of Science One 8 art. e77354.

Loope, K.J., Baty, J.W., Lester, P.J. & Wilson Rankin, E.E. 2019: Pathogen shifts in a honeybee predator following the arrival of the *Varroa* mite. – Proceedings of the Royal Society B-Biological Sciences 286: art. 20182499.

Manfredini, F., Shoemaker, D. & Grozinger, C.M. 2016: Dynamic changes in host-virus interactions associated with colony founding and social environment in fire ant queens (*Solenopsis invicta*). – Ecology and Evolution 6: 233-244.

Maori, E., Lavi, S., Mozes-Koch, R., Gantman, Y., Peretz, Y., Edelbaum, O., Tanne, E. & Sela, I. 2007: Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra- and inter-species recombination. – Journal of General Virology 88: 3428-3438.

Mason, A.P., Smilanich, A.M. & Singer, M.S. 2014: Reduced consumption of protein-rich foods follows immune challenge in a polyphagous caterpillar. – The Journal of Experimental Biology 217: 2250-2260.

Oi, D. & Valles, S. 2009: Fire ant control with entomopathogens in the USA. In: Hajek, A.E., Glare, T.R. & O'Callaghan, M. (Eds.): Use of microbes for control and eradication of invasive arthropods. – Springer Science, New York, NY, pp. 237-258.

Oi, D., Valles, S., Porter, S., Cavanaugh, C., White, G. & Henke, J. 2019: Introduction of fire ant biological control agents into the Coachella Valley of California. – Florida Entomologist 102: 284-286.

Oi, D., Porter, S.D. & Valles, S.M. 2015: A review of the biological control of fire ants (Hymenoptera: Formicidae). – Myrmecological News 21: 101-116.

Olendraite, I., Brown, K., Valles, S.M., Firth, A.E., Chen, Y., Guérin, D.M.A., Hashimoto, Y., Herrero, S., de Miranda, J.R. & Ryabov, E.V. 2019: ICTV virus taxonomy profile: Polycipiviridae. – Journal of General Virology 100: 554-555.

Olendraite, I., Lukhovitskaya, N.I., Porter, S.D., Valles, S.M. & Firth, A.E. 2017: Polycipiviridae: a proposed new family of polycistronic picorna-like RNA viruses. – Journal of General Virology 98: 2368-2378.

Payne, A.N., Shepherd, T.F. & Rangel, J. 2020: The detection of honey bee (*Apis mellifera*)-associated viruses in ants. – Scientific Reports 10: art. 2923.

Porter, S.D., Gavilanez-Slone, J.M. & Valles, S.M. 2016a: Solenopsis invicta virus 3: infection tests with adult honey bees (Hymenoptera: Apidae). – Florida Entomologist 99: 729-733.

Porter, S.D., Valles, S.M. & Oi, D.H. 2013: Host specificity and colony impacts of the fire ant pathogen, Solenopsis invicta virus 3. – Journal of Invertebrate Pathology 114: 1-6.

Porter, S.D., Valles, S.M. & Pereira, R.M. 2016b: Scavenging crickets (Orthoptera: Gryllidae) transmit *Solenopsis invicta* virus 3 to red imported fire ant (Hymenoptera: Formicidae) colonies. – Florida Entomologist 99: 811-812.

Porter, S.D., Valles, S.M., Wild, A.L., Dieckmann, R. & Plowes, N.J.R. 2015: Solenopsis invicta virus 3: further host-specificity tests with native *Solenopsis* ants (Hymenoptera: Formicidae). – Florida Entomologist 98: 122-125.

Posada-Florez, F., Childers, A.K., Heerman, M.C., Egekwu, N.I., Cook, S.C., Chen, Y., Evans, J.D. & Ryabov, E.V. 2019: Deformed wing virus type A, a major honey bee pathogen, is vectored by the mite *Varroa destructor* in a non-propagative manner. – Scientific Reports 9: art. 12445.

Ribière, M., Ball, B.V. & Aubert, M.F.A. 2008: Natural history and geographic distribution of honey bee viruses. In: Aubert, M.F.A., Ball, B.V., Fries, I., Milani, N. & Moritz, R.F.A. (Eds.): Virology and the honey bee. – EC Publications, Brussels, pp. 15-84.

Ribière, M., Olivier, V. & Blanchard, P. 2010: Chronic bee paralysis: a disease and a virus like no other? – Journal of Invertebrate Pathology 103 Supplement 1: S120-S131.

Sakuna, K., Elliman, J. & Owens, L. 2017: Discovery of a novel *Picornavirales*, chequa iflavirus, from stressed redclaw crayfish (*Cherax quadricarinatus*) from farms in northern Queensland, Australia. – Virus Research 238: 148-155.

Schläppi, D., Chejanovsky, N., Yanez, O. & Neumann, P. 2020: Foodborne transmission and clinical symptoms of honey bee viruses in ants *Lasius* spp. – Viruses 12 art. 321.

Schläppi, D., Lattrell, P., Yanez, O., Chejanovsky, N. & Neumann, P. 2019: Foodborne transmission of deformed wing virus to ants (*Myrmica rubra*). – Insects 10 art. 394.

Schmid-Hempel, P. 1998: Parasites in social insects. – Princeton University Press, Princeton, NJ, 392 pp.

Schroeder, D.C. & Martin, S.J. 2012: Deformed wing virus: the main suspect in unexplained honeybee deaths worldwide. – Virulence 3: 589-591.

Sebastien, A., Lester, P.J., Hall, R.J., Wang, J., Moore, N.E. & Gruber, M.A. 2015: Invasive ants carry novel viruses in their new range and form reservoirs for a honeybee pathogen. – Biology Letters 11: art. 20150610.

Shi, M., Lin, X.D., Tian, J.H., Chen, L.J., Chen, X., Li, C.X., Qin, X.C., Li, J., Cao, J.P., Eden, J.S., Buchmann, J., Wang, W., Xu, J., Holmes, E.C. & Zhang, Y.Z. 2016: Redefining the invertebrate RNA virosphere. – Nature 540: 539-543.

Shikano, I. & Cory, J.S. 2016: Altered nutrient intake by baculovirus-challenged insects: Self-medication or compensatory feeding? – Journal of Invertebrate Pathology 139: 25-33.

Steiger, U., Lamparter, H.E., Sandri, C. & Akert, K. 1969: Virus-like particles in cytoplasm of neurons and glial cells of wood ants. – Archives of Virology 26: 271-282. (in German)

Stroeymeyt, N., Grasse, A.V., Crespi, A., Mersch, D.P., Cremer, S. & Keller, L. 2018: Social network plasticity decreases disease transmission in a eusocial insect. – Science 362: 941-945.

Tufts, D.M., Hunter, W.B. & Bextine, B. 2014: Solenopsis invicta virus (Sinv-1) infection and insecticide interactions in the red imported fire ant (Hymenoptera: Formicidae). – Florida Entomologist 97: 1251-1254.

Valles, S.M. 2012: Positive-strand RNA viruses infecting the red imported fire ant, *Solenopsis invicta*. – Psyche 2012: art. 821591.

Valles, S.M., Bell, S. & Firth, A.E. 2014a: Solenopsis invicta virus 3: Mapping of structural proteins, ribosomal frameshifting, and similarities to Acyrthosiphon pisum virus and kelp fly virus. – Public Library of Science One 9: art. e93497.

Valles, S.M. & Hashimoto, Y. 2009: Isolation and characterization of *Solenopsis invicta* virus 3, a new positive-strand RNA virus infecting the red imported fire ant, *Solenopsis invicta*. – Virology 388: 354-361.

Valles, S.M. & Oi, D.H. 2014: Successful transmission of Solenopsis invicta virus 3 to field colonies of *Solenopsis invicta* (Hymenoptera: Formicidae). – Florida Entomologist 97: 1244-1246.

Valles, S.M., Oi, D.H., Becnel, J.J., Wetterer, J.K., LaPolla, J.S. & Firth, A.E. 2016: Isolation and characterization of Nylanderia fulva virus 1, a positive-sense, single-stranded RNA virus infecting the tawny crazy ant, *Nylanderia fulva*. – Virology 496: 244-254.

Valles, S.M., Oi, D.H. & Porter, S.D. 2010: Seasonal variation and the co-occurrence of four pathogens and a group of parasites among monogyne and polygyne fire ant colonies. – Biological Control 54: 342-348.

Valles, S.M. & Porter, S.D. 2013: Procedures to mitigate the impact of Solenopsis invicta virus 3 in fire ant (Hymenoptera: Formicidae) rearing facilities. – Florida Entomologist 96: 252-254.

Valles, S.M. & Porter, S.D. 2015: Dose response of red imported fire ant colonies to Solenopsis invicta virus 3. – Archives of Virology 160: 2407-2413.

Valles, S.M. & Porter, S.D. 2019: Influence of temperature on the pathogenicity of Solenopsis invicta virus 3. – Journal of Invertebrate Pathology 166: 107217.

Valles, S.M., Porter, S.D. & Calcaterra, L.A. 2018: Prospecting for viral natural enemies of the fire ant *Solenopsis invicta* in Argentina. – Public Library of Science One 13: art. e0192377.

Valles, S.M., Porter, S.D., Choi, M.-Y. & Oi, D.H. 2013: Successful transmission of Solenopsis invicta virus 3 to *Solenopsis invicta* fire ant colonies in oil, sugar, and cricket bait formulations. – Journal of Invertebrate Pathology 113: 198-204.

Valles, S.M., Porter, S.D. & Firth, A.E. 2014b: Solenopsis invicta virus 3: pathogenesis and stage specificity in red imported fire ants. – Virology 460-461: 66-71.

Valles, S.M. & Rivers, A.R. 2019: Nine new RNA viruses associated with the fire ant *Solenopsis invicta* from its native range. – Virus Genes 55: 368-380.

Valles, S.M., Strong, C.A., Dang, P.M., Hunter, W.B., Pereira, R.M., Oi, D.H., Shapiro, A.M. & Williams, D.F. 2004: A picorna-like virus from the red imported fire ant, *Solenopsis invicta*: initial discovery, genome sequence, and characterization. – Virology 328: 151-157.

Valles, S.M., Strong, C.A. & Hashimoto, Y. 2007a: A new positive-strand RNA virus with unique genome characteristics from the red imported fire ant, *Solenopsis invicta*. – Virology 365: 457-463.

Valles, S.M., Strong, C.A., Oi, D.H., Porter, S.D., Pereira, R.M., Vander Meer, R.K., Hashimoto, Y., Hooper-Bui, L.M., Sanchez-Arroyo, H., Davis, T., Karpakakunjaram, V., Vail, K.M., Fudd Graham, L.C., Briano, J.A., Calcaterra, L.A., Gilbert, L.E., Ward, R., Ward, K., Oliver, J.B., Taniguchi, G. & Thompson, D.C. 2007b: Phenology, distribution, and host specificity of *Solenopsis invicta* virus-1. – Journal of Invertebrate Pathology 96: 18-27.

Valles, S.M., Varone, L., Ramírez, L. & Briano, J. 2009: Multiplex detection of Solenopsis invicta viruses -1, -2, and -3. – Journal of Virological Methods 162: 276-279.

Viljakainen, L., Holmberg, I., Abril, S. & Jurvansuu, J. 2018a: Viruses of invasive Argentine ants from the European main supercolony: characterization, interactions and evolution. – Journal of General Virology 99: 1129-1140.

Viljakainen, L. & Jurvansuu, J. 2020: Discovery and analysis of RNA viruses in insects. In: Sandrelli, F. & Tettamanti, G. (Eds.): Immunity in insects. – Springer US, New York, NY, pp. 191-200.

Viljakainen, L., Jurvansuu, J., Holmberg, I., Pamminger, T., Erler, S. & Cremer, S. 2018b: Social environment affects the transcriptomic response to bacteria in ant queens. – Ecology and Evolution 8: 11031-11070.

Wu, Q., Luo, Y., Lu, R., Lau, N., Lai, E.C., Li, W.X. & Ding, S.W. 2010: Virus discovery by deep sequencing and assembly of virus-derived small silencing RNAs. – Proceedings of the National Academy of Sciences of the United States of America 107: 1606-1611.

Yang, C.-C., Yu, Y.-C., Valles, S.M., Oi, D.H., Chen, Y.-C., Shoemaker, D., Wu, W.-J. & Shih, C.-J. 2010: Loss of microbial (pathogen) infections associated with recent invasions of the red imported fire ant *Solenopsis invicta*. – Biological Invasions 12: 3307-3318.